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Molybdenum plays an important biological role as a micronutrient for plants and animals. At high levels it can be toxic to animals. While concentrations in surface waters are generally less than 5 µgMo/L, concentrations as high as 500 µgMo/L have been reported in some drinking waters. Concentrations in water greater than 20 µgMo/L are almost certainly anthropogenic.

The average human intake via food for the United States is 170 µgMo/day while the average intake via drinking water is less than 5 µgMo/day. While no adverse health effects have been reported in the United States, there are reports in the Russian and Indian literature of both biochemical and clinical effects in humans at intakes ranging from 1 to 10 mgMo/day. Rapid urinary excretion appears to provide considerable protection at intakes less than 1 mgMo/day. This report reviews the data on molybdenum as it relates to the effects of its occurrence in drinking water.

The report also reviews the results of an interdisciplinary study carried out by the authors. The authors recommend a guideline of 50 µgMo/L for the maximum concentration in drinking water.

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HUMAN HEALTH EFFECTS OF MOLYBDENUM IN DRINKING WATER

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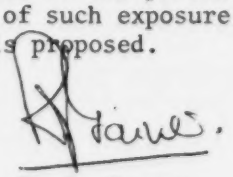
FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report provides an assessment of the present environmental exposure to molybdenum and the health effects of such exposure. A guideline for maximum concentration in drinking water is proposed.



R. J. Garner
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ABSTRACT

Molybdenum is a trace element which occurs widely in nature and plays an important role in industrial society. The primary industrial use of molybdenum is as an alloy in steels. Major releases to the environment have been associated with several industries including molybdenum mining and milling, uranium mining and milling, and oil refining.

Molybdenum also plays an important biological role as a micronutrient for plants and animals. At high levels it can be toxic to animals. While concentrations in surface waters are generally less than 5 µgMo/L, concentrations as high as 500 µgMo/L have been reported in some drinking waters. Concentrations in water greater than 20 µgMo/L are almost certainly anthropogenic. Conventional wastewater and water treatment technologies are ineffective in the removal of molybdenum.

The average human intake via food for the United States is 170 µgMo/day while the average intake via drinking water is less than 5 µgMo/day. While no adverse health effects have been reported in the United States, there are reports in the Russian and Indian literature of both biochemical and clinical effects in humans at intakes ranging from 1 to 10 mgMo/day. Rapid urinary excretion appears to provide considerable protection at intakes less than 1 mgMo/day. This report reviews the data on molybdenum as it relates to the effects of its occurrence in drinking water.

The report also reviews the results of an interdisciplinary study carried out by the authors. The authors recommend a guideline of 50 µgMo/L for the maximum concentration in drinking water.

This report was submitted in fulfillment of Grant No. R-803645 by the Environmental Trace Substances Research Program, University of Colorado under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from April 7, 1975 to September 30, 1978, and work was completed as of September 30, 1978.

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SECTION 1

INTRODUCTION

Two-thirds of the elements occurring in the earth's crust are more abundant than molybdenum, yet it is among the fifteen trace elements that are essential to plants and animals. Molybdenum is also economically important as a component of metal alloys, fertilizers, catalysts, and anti-corrosive agents. Its rapidly growing production and use represents a potential for increased release and distribution in the environment. The purpose of this report is to describe the human health effects that water-borne molybdenum may have.

In spite of its relatively low natural abundance the amount of soil molybdenum available for plant uptake appears to be sufficient in most areas. Geochemical anomalies leading to molybdenum deficiencies in plants have been described; however, nutritional deficiencies in humans have not been documented. Therefore, the human nutritional requirement is probably low, or modern food distribution practices tend to ameliorate any deficiencies in locally produced foods.

Molybdenum is similar to other essential trace elements in that it exhibits a detrimental biochemical effect when the animal's intake exceeds the optimum amount. The symptoms of molybdenum toxicity and the dietary concentration at which symptoms occur varies with species. Much has been published about the effects of excessive molybdenum consumption by cattle, and the molybdenum-induced disease in ruminants, molybdenosis, has been well documented. However, little is known about the human health effects. The following sections review the existing literature relevant to the assessment of the human health effects of exposure to anomalously high concentrations of molybdenum. This report also includes the results obtained by the authors in a study of human exposures to molybdenum in water, food, and air.

Most surface and ground waters contain about 1 µgMo/L. Stream waters draining undisturbed molybdenum mineral deposits are also low. Therefore, waters containing more than 10 to 20 µgMo/L are usually associated with human activities such as mining, upgrading, or other industrial processing. Several municipal water supplies in Colorado exhibit anomalously high molybdenum concentrations. This report describes the measurement of several biochemical parameters on selected groups of individuals who receive their drinking water from these sources. The study also includes an extensive food sampling program to determine the molybdenum intake derived from food consumption.

Since relatively few humans are presently exposed to high molybdenum concentrations in food or water, we have also included studies of humans who have been industrially exposed to molybdenum compounds. These data are

supplemented with relevant animal studies. The conclusions presented in Section 2 are therefore based upon the best available data from the literature, and the authors' own work on both humans and animals.

Brief descriptions of the chemistry of molybdenum, its production, use, and environmental fate precede the discussion of the biochemistry and biological effects. The reference list does not cite all of the published work on the subject. However, it represents the bulk of the relevant work upon which the conclusions and recommendations are based.

COMMENT ON CONCENTRATION UNITS

Concentrations of trace constituents in solid samples are reported in parts per million (ppm). This unit is equivalent to micrograms of analyte per gram of sample. Concentrations of an analyte in liquid samples are reported in micrograms analyte per liter of solution. This convention is convenient since most natural waters contain less than 20 µgMo/L. Concentrations of ten to one thousand times the "natural" levels are only observed as a result of anthropogenic activities. In order to emphasize the order of magnitude differences between natural and contaminated waters large concentrations have not been converted to the more common multiples such as mg/L.

However, the units of mg/L are used in portions of Sections 7 and 8 since the Laboratory Studies involved concentrations of thousands of micrograms molybdenum per liter of water. These concentrations are therefore converted to milligrams per liter to facilitate the reading of the text.

It should again be emphasized that concentrations of milligrams per liter in waters are several orders of magnitude above natural levels.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

While conclusive evidence that molybdenum is required by humans is lacking, there is general agreement that it should be considered as one of the essential trace elements. The absence of any documented deficiencies in humans indicates that the required level is much less than the average intake of 180 µgMo/day in the United States.

Except for industrial workers, the intake of molybdenum is almost entirely via food and beverages, including water. Abnormally high food intakes, as high as 10 to 15 mgMo/day, have been documented in India and the U.S.S.R. and are suspected in Turkey. Because of the interregional distribution system in the United States, it is unlikely that molybdenum toxicity will be encountered in humans due to food intake. There could be exceptions where significant parts of the diet come from one particular geographical area or for individuals on a limited diet.

Natural molybdenum concentrations in ground and surface waters are rarely more than 10 to 20 µgMo/L. Concentrations significantly higher than these levels are almost certainly due to industrial contamination. Since conventional wastewater and water treatment facilities remove very little (0 to 20%) molybdenum, drinking water concentrations will be close to those of the untreated source.

Several industries have effluents which have high concentrations of molybdenum. These include molybdenum mining and milling, molybdenum smelting, uranium mining and milling, copper mining and milling, shale oil production, and coal-fired power plants. The aqueous effluents from these industries have molybdenum concentrations ranging from 100 to 800,000 µgMo/L. The most frequently observed environmental impact is molybdenum toxicity in cattle.

There is great species variation in the susceptibility of animals to molybdenum toxicity. Acute toxicity in other than the laboratory setting has only been seen in cattle and sheep. Cattle are by far the least tolerant of molybdenum, while sheep are somewhat more tolerant. Rats, guinea pigs, and poultry are more tolerant than cattle and sheep, and less tolerant than pigs. Symptoms of molybdenum toxicity also vary with species. In the case of cattle, severe diarrhea is a common symptom. Other animals may suffer from loss of weight, sterility, anemia, connective tissue lesions, and pathological changes in the liver and kidney depending on the species and dose. The only clinical symptom described in humans is a gout-like disease. Laboratory and

field studies indicate that molybdenum is a biological antagonist to copper. Thus, the usual mechanism of molybdenum toxicity is the induction of copper deficiency. Symptoms in cattle and other animals can often be reversed by the addition of supplemental copper to the diet.

While there are people in the United States with water supplies containing greatly elevated concentrations of molybdenum, the corresponding daily intakes are still at least an order of magnitude less than those people in the U.S.S.R. who were described as exhibiting a gout-like disease. Industrial workers in the United States are exposed to intakes that can be as high as 50 mgMo/day without violating OSHA standards.

Molybdenum in the diet and in water is readily absorbed and rapidly excreted. The rapid excretion, primarily in urine, provides an effective mechanism for regulating molybdenum concentrations in blood and presumably other tissues. In our study of humans having water supplies containing as much as 200 µgMo/L, we found that while urinary concentrations were increased, serum molybdenum concentrations remained normal. No changes in copper metabolism were observed. However, Deosthale and Gopalan (1) have observed an increase in daily urinary copper excretion in human subjects receiving 500 to 1,000 µgMo/day in their diets.

In summary, no biochemical or clinical effects were observed in humans whose water supplies contained up to 50 µgMo/L. Increased urinary excretion of molybdenum was observed in humans whose water supplies contained 50 to 200 µgMo/L. Deosthale and Gopalan (1) observed increased copper excretion in humans having daily intakes of 500 to 1,500 µgMo/day, but they did not observe any changes in uric acid excretion. In a study of molybdenum workers exposed to a minimum daily intake of 10 mgMo we found greatly increased blood and urine levels of molybdenum. We also found significant increases in uric acid excretion, but these levels were still within a normal range for humans. We also found a significant increase in serum ceruloplasmin compared to normals, consistent with the results of Deosthale and Gopalan (1). In addition, we found an increased xanthine oxidase activity. However, these levels were still within a normal range for humans. Kovalskii and others (2), studied a human population receiving 10 to 15 mgMo/day. They found greatly increased uric acid levels, decreased copper excretion, and a high incidence of a gout-like disease. They postulated that the increased uric acid excretion and gout-like disease were due to increased xanthine oxidase (a molybdenum-containing enzyme) which was in turn due to the abnormal molybdenum intake.

RECOMMENDATIONS

In view of the absence of any documented human cases of molybdenum deficiency, it seems likely that the average daily intake of 180 µg Mo is well above the minimum daily requirement. All micronutrients are characterized by a range of deficiency, a range of sufficiency, and beyond these, toxicity. Since the exact size of the range of sufficiency is unknown, it would seem prudent to avoid large increases beyond the average dietary intake of 180 µgMo/day. On the other hand, clinical effects have been reported at 10,000 to 15,000 µgMo/day and biochemical effects in the range of 500 to 10,000 µgMo/

day. The "no-effect" level cannot be pinpointed with certainty, but we feel it is probably not less than 500 µgMo/day. A concentration of 50 µgMo/L in drinking water would contribute about 100 µgMo/day to the total diet which would make the average total daily intake 280 µgMo/day. Even for 15 to 17 year old males, the group having the largest average dietary intake at 250 µgMo/day, the total would be 350 µgMo/day. Since no differences were seen between humans on 1 µgMo/L and on 50 µgMo/L, it appears that 50 µgMo/L represents a "safe" level in drinking water. (A supplementary method for calculating this level is found in Appendix A.)

Thus, we believe 50 µgMo/L represents a useful guideline for drinking water. Our data indicate that somewhat higher levels can probably be tolerated, at least for short periods of time. Where levels above 50 µgMo/L occur it is almost certain to be as the result of industrial contamination. While such concentrations are not often encountered, recent studies by ourselves and others show that some communities in different regions of the United States do have finished water supplies containing more than 50 µgMo/L. Other such communities will be encountered in the future.

It is not likely that a drinking water standard for molybdenum is required. But we believe that 50 µgMo/L represents a prudent guideline.

In order for a recommended level to be useful, the analytical techniques used or recommended by regulatory agencies must be capable of accurate and precise measurements at or, preferably, below that level. The present technique recommended by E.P.A. (Methods for Chemical Analysis of Water and Wastes; EPA 625/6-74-003) and used for monitoring purposes (e.g., in N.P.D. E.S. compliance) is not adequate. This flame atomic absorption technique does not have a detection limit sufficiently low to monitor most drinking waters. The method is also highly susceptible to the interferences presented by most industrial effluent matrices. There are other methods of analysis which have detection limits in the range of 1 to 10 µgMo/L which could be validated for routine use (e.g., atomic absorption with electrothermal atomization, colorimetric methods). Direct flame atomic absorption without preconcentration and/or extraction to separate the analyte from interferences is not likely to provide accurate analyses. In view of the guideline recommended above and the existing recommended level for irrigation water (10 µgMo/L for continuous irrigation), we strongly recommend that EPA adopt a different analytical technique for general use. Two such techniques are described in Appendices B and C.

SECTION 3

CHEMICAL PROPERTIES

GENERAL CHEMISTRY

The chemistry of molybdenum is so extensive that it is necessary to limit the discussion to chemical and physical properties that are important under conditions which occur naturally in the environment or in biological systems.

Molybdenum, atomic number 42, is a transition element with the outermost electronic structure $4d^5 5s^1$. It exhibits oxidation states from 2- to 6+. However, the oxidation states below 2+ are generally found only in organometallic compounds. Of the remaining oxidation states, only 3+, 4+, 5+, and 6+ are important in aqueous solution. Oxides and sulfides of these oxidation states are the principal solid inorganic species which occur naturally. Crystalline oxides and sulfides are well characterized and are widely used industrially. Molybdenum also forms the tetrahedral poly atomic anion molybdate, MoO_4^{2-}, and isopolyanions such as $Mo_7O_{24}^{6-}$. These anions form salts with a variety of cations and these compounds are used in industrial applications. Some of the important naturally occurring compounds are the minerals: molybdenite, powellite, wulfenite, ferrimolybdite, ilsemannite. Table 1 shows the chemical formulas for these compounds. Most of these minerals are only slightly soluble at natural pHs and oxidation-reduction conditions.

The relative stabilities for naturally occurring minerals in equilibrium with water and dissolved species can be illustrated by using theoretical Eh-pH diagrams. The species oxidation potential, relative to the hydrogen electrode, is plotted as a function of pH. Such a diagram shows the regions of stability for the various species under consideration. Figure 1 shows an Eh-pH diagram for the predominant aqueous molybdenum species (3). The shaded area represents the range of natural Eh-pH characteristics which have been measured for shallow ground waters and fresh waters (4). From this diagram it can be seen that most natural waters exhibit conditions under which MoO_4^{2-} would be the principal stable species. At pHs below 5 and under more oxidizing conditions the hydrogen molybdate anion ($HMoO_4^-$) is expected to be the predominant species. Such Eh-pH conditions are likely to be found in some acid mine drainages or industrial effluents. Similarly, at very low pHs and under slightly less oxidizing conditions, the cationic MoO_2^+ is the principal species present.

When there are significant concentrations of other ionic species such as iron, calcium, sulfate, etc., these species can also be included in the Eh-pH diagram. The presence of some of these species can significantly alter the aqueous molybdenum chemistry. However, the following generalization remains:

TABLE 1. SOME PHYSICAL PROPERTIES OF MOLYBDENUM

Color	metallic silver
Atomic number	42
Atomic mass	95.94
Atomic radius	1.40A
Density	10.28 g/cm^3 (20°C)
Melting point	2620 ± 10°C
Boiling point	4825°C

Formulas of common minerals:

Molybdenite	MoS_2
Powellite	$CaMoO_4$
Wulfenite	$PbMoO_4$
Ferrimolybdite	$Fe_2O_3 \cdot 3.52MoO_3 \cdot 10.4H_2O$
Ilsemannite	Mo_3O_8

the principal dissolved molybdenum species in the natural environment is molybdate. For a more detailed description of molybdenum mineral solubility and aqueous geochemistry the reader is directed to the work of D. Kaback (3).

BIOINORGANIC CHEMISTRY

In order to understand the biochemistry of the molybdenum-bearing enzymes it is helpful to examine the chemical interactions between molybdenum and biologically important molecules. While the exact nature of the molybdenum-protein interactions is incompletely understood, several generalizations regarding these interactions can be made. The 3+, 4+, 5+, and 6+ oxidation states of molybdenum are the most important in biological systems (5). A protein can bind to a transition element through oxygen, sulfur, or nitrogen. In general, higher oxidation states lead to oxygen binding while lower oxidation states favor sulfur or nitrogen binding. Oxygen and sulfur are favored over nitrogen as ligands. This is partly due to the ability of oxygen and sulfur to partially neutralize the positive charge of the metal atom (6). The neutral nitrogen in most ligands does not afford this added stabilization. The coordination number of molybdenum with most organic ligands is five or six. This is in contrast to other biologically important transition elements such as iron, copper, and zinc which are four coordinate (6).

There is considerable evidence that the molybdenum in xanthine oxidase and other enzymes is bound through the mercapto group of cysteine residue (7). Both Mo (V) and Mo (VI) are known to form complexes with histidine. The molybdenum enzymes are catalysts in oxidation-reduction reactions. Molybdenum

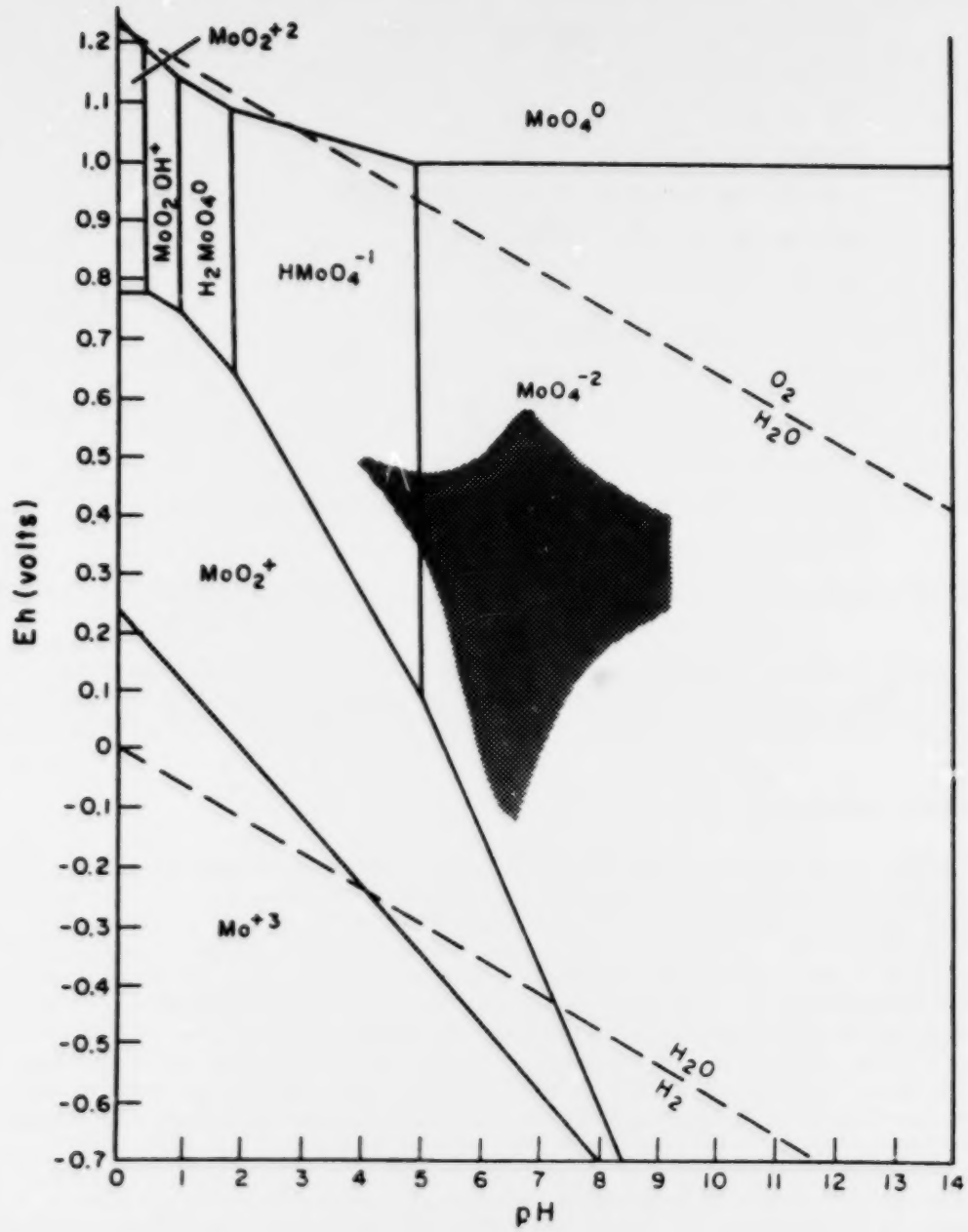

Figure 1. Eh-pH diagram of areas of predominance of aqueous species of molybdenum [after Kaback (3)]. Shaded areas shows Eh-pH characteristics of fresh waters [after Baas-Becking et. al. (4)].

frequently occurs in enzymes where there are other electron carriers such as iron/sulfur, iron/heme, and iron/flavin. Molybdenum participates in electron transfer, and may be preferred over other metals in this role since it has stable oxidation states from 3+ to 6+ and can undergo multiple electron transfers (6). The biochemistry and metabolism of molybdenum will be discussed in Section 7.

SECTION 4

MEASUREMENT TECHNIQUES

SAMPLING

Waters, geological solids, plants, animal tissues, and biological fluids all have complex chemical characteristics which must be considered before an adequate sampling protocol can be devised. The choice of a sampling procedure depends upon field sampling conditions, the chemical characteristics of the material being sampled, and the purpose to which the analyses will be put. Since the purpose of this report is to present data on the human health effects of molybdenum in drinking water, we shall limit our discussion to sampling methods which have a direct bearing on this study. Therefore, water sampling and analysis will be discussed in detail.

No single method of collection and treatment is suitable for all types of water samples. Each chemical constituent of water may affect the stability of other constituents. Preservation methods which ensure quantitative recovery of molybdenum may not effectively preserve other trace elements. Therefore, it is essential that the adequacy of the sample treatment be confirmed for each element of interest before extensive sampling is undertaken. The following section describes the results of a study of sampling factors which affect the validity of molybdenum analyses.

Adsorption of trace elements on the surface of sample walls is potentially the most serious source of error in water sampling (8). The extent of molybdenum adsorption, at equilibrium, depends upon the container material, ionic strength, pH, and molybdenum concentration (9). The maximum adsorption of molybdenum on polyethylene occurs at about pH 4. At this pH about 25% is adsorbed from an aqueous solution of 5 µgMo/L. About 3% adsorption occurs at 100 µgMo/L. Adsorption is variable and greater when iron is present in solution at 2 mg/L. Coprecipitation or coadsorption with hydrated ferric oxides can severely reduce the recovery of molybdenum from improperly treated samples. Glass, linear, and cross-linked polyethylene containers are suitable for collection and storage of water samples when the samples are acidified to less than pH 2. Samples stored at pH 2 in these bottles for three years showed quantitative recoveries. The light weight and unbreakable nature of polyethylene containers makes them convenient for field collection and shipping. Bottles may be reused if they are thoroughly cleaned with a detergent wash followed by a dilute nitric and/or hydrochloric acid wash. While no molybdenum contamination was found in unwashed new bottles, several other trace elements present serious contamination problems. Acid washing of new bottles is recommended when other trace elements are to be determined (10).

The need for sample filtration depends upon how the analyses will be used. When human or animal intake is to be determined, filtration may not be appropriate since molybdenum present in suspended colloidal material is consumed along with dissolved molybdenum. The tradition of filtering samples through 0.45 micron membrane filters was based upon the desire to remove biological materials; however, this practice complicates the interpretation of the existing literature. This poorly justified filtration practice has become the arbitrary criterion for distinguishing between "dissolved" and "suspended" species. Experienced workers are aware of the tendency of membrane filters to retain species smaller than the nominal filter pore size. The effective pore size of these filters is reduced as the membrane becomes clogged. In addition, membranes heavily loaded with colloidal iron hydroxides can strongly adsorb some dissolved species. These limitations should be considered when one interprets data obtained on filtered samples.

Analyses obtained on unfiltered natural water samples can also complicate interpretation. This is particularly true of samples which contain sediment and/or large amounts of suspended material. These materials can serve as sinks for adsorption and/or sources of elemental contamination through desorption or dissolution. Samples obtained from rapidly flowing streams often carry large amounts of material which settle out under less turbulent flow conditions. Sampling these waters requires great care. Adequate molybdenum analyses may be obtained by allowing these waters to settle in the sample bottle. Aliquots of the supernatant may be used for analysis. Such samples should not be acidified since the added acid may dissolve some of the solid material. Samples should be analyzed as soon as possible after collection.

Most drinking and natural waters can be analyzed without filtration since most of the molybdenum present remains in solution as the molybdate or hydrogen molybdate species. Extremes of pH or high concentrations of other species such as iron are not likely to be found in most drinking waters. Figure 1, the Eh-pH diagram shown in Section 3 of this report, illustrates the stability fields of the principal aqueous species. Furthermore, most of the naturally occurring molybdenum-bearing minerals which may occur in sediments are not highly soluble under the chemical conditions of natural waters.

Biological growth in stored samples can also change the trace element content of stored samples. Since most household tap waters are chlorinated no pretreatment is necessary. However, untreated waters may be treated with bacteriostatic agents to retard biological activity. One mL of chloroform per liter of sample is sufficient to prevent growth and does not interfere with the analysis.

Freezing of samples is frequently recommended for preservation of trace elements in waters. Winter collection of natural waters often precludes proper pretreatment of samples in the field before they freeze. Since freezing can irreversibly alter the stability of some trace elements (11), a study to determine the effects of freezing of molybdenum in waters was performed. This study showed that freezing does not affect the analytical concentration of molybdenum. Treatment in the field is always the preferred method of sample preservation; however, samples which remain frozen until analysis can later be treated with acid in the laboratory to reversibly recover molybdenum.

When iron is present at concentrations greater than 2 mgFe/L poor iron recovery is obtained which in turn affects the molybdenum recovery from unacidified iron rich water samples. A comprehensive survey of the current literature on sample handling is available in an NBS Technical Note (12).

ANALYSES

Quantitative analysis of molybdenum as a trace constituent has been attempted by a wide variety of techniques. While emission spectroscopy, x-ray fluorescence, and neutron activation have been successfully used in the analysis of aqueous samples, the cost and availability of the instrumentation required by these methods precludes their use by the majority of analytical laboratories responsible for water quality monitoring. Therefore, colorimetric and atomic absorption spectrophotometry are the most widely used analytical detection techniques today.

Colorimetric Methods

Molybdenum forms several stable colored complexes which can be used for detection and quantitative analysis of aqueous molybdenum. However, these complexes have low molar absorptivities and the presence of other trace elements can interfere with the analysis. Improved sensitivity and specificity can be obtained by using a solvent extraction technique to concentrate the colored analyte and to separate it from potential interferents.

The widely used thiocyanate colorimetric method makes use of the amber-colored molybdenum-thiocyanate complex. In this method molybdenum is reduced in acid medium by tin(II) ion. Addition of thiocyanate ion yields the amber molybdenum-thiocyanate complex which is readily extracted into a polar organic solvent. Ethers, alcohols, and ketones, which are immiscible with water, are used for the extraction. The intensity of the amber colored complex in standards and unknowns may then be determined using a spectrophotometer. The detection limit and sensitivity* of this method depends upon the relative volumes of sample and organic extractant; however, the method is capable of detecting molybdenum in water at concentrations as low as 1 µgMo/L. Several modifications of this technique have been published (13-16).

Another widely used colorimetric method is the dithiol method. Toluene-3, 4-dithiol reacts with hexavalent molybdenum in hydrochloric acid solution to form a green complex which is extractable with chloroform, carbon tetrachloride, or other organic solvents. This method is also capable of detecting molybdenum concentrations in the low µgMo/L range. Separation using ion-

---

* Detection limit = the concentration of an element which will shift the absorbance (or emission) signal an amount equal to the peak-to-peak noise of the baseline (or background signal).

Sensitivity = the concentration of an element which produces an absorbance of 0.0044 (1% absorption).

exchange resins have also been used to preconcentrate the sample and to remove interferents (17,18). Modifications and various improvements are described in several references (14, 19-23).

Atomic Absorption Spectrophotometry

For most metal elements atomic absorption spectrophotometry (A.A.S.) is the preferred method of analysis because of its speed and simplicity. However, accurate molybdenum analyses by A.A.S. can be difficult and time consuming. Molybdenum is one of several metals which forms refractory oxides in flames. This tendency can be minimized by the use of a nitrous oxide-acetylene reducing flame. However, even in reducing flames, the propensity to remain in an oxidized form is enhanced by the presence of other interfering elements. One study of molybdenum absorption in flames has shown that 46 added ionic species affect the signal intensity (24). The magnitude of the interference depends upon the concentration of the interferent. Therefore, complete separation of molybdenum from potential interferents is necessary to insure accurate analyses. Adequate separations can be accomplished by ion exchange separation or by complexation-extraction techniques (14,16,25,26). The complexation-extraction procedures used in the colorimetric methods have been used for analysis of molybdenum by flame A.A.S. In these procedures the organic phase bearing the molybdenum complex is aspirated into the flame for detection and quantification (25). These techniques tend to be more time consuming than the colorimetric procedures.

Another difficulty encountered with the A.A.S. determination of molybdenum is its low practical sensitivity of about 500 µgMo/L. Even with large preconcentration factors the detection limit of about 10 µgMo/L is insufficient for many waters. Direct analysis of natural waters without preconcentration is not possible since the detection limit of the method is between 50 and 100 µg/L. Most natural and drinking waters must be concentrated by a factor of at least ten before adequate precision can be achieved.

Flamesless A.A. techniques have recently become more widely used. In this technique atomization is accomplished by introducing a small aliquot (usually 10-50 µL) of the aqueous sample into a graphite furnace or cup. The graphite furnace or cup is resistance heated to about 3,000^{o}C by passing an electric current through it. The electrothermally induced atomization process replaces the flame induced atomization of conventional A.A.S. This method is much more sensitive than flame A.A. techniques. Detection limits of 1 µg/L may be achieved without preconcentrating the sample. However, this method is also susceptible to interferences. Several ions severely suppress the absorption signal and high dissolved salt concentrations make the detection limit poorer. However, since most tap waters are low in dissolved solids, they may be analyzed directly by this method. With some modifications the method is applicable to the determination of molybdenum in biological materials when low concentrations and only small samples are available (27).

Other Methods

Emission spectrography (E.S.) has been used for the analysis of molybdenum in solid samples. However, water analysis by E.S. requires precipitation

of molybdenum as a complex which can be dry mounted in the instrument electrodes. This method is tedious and time consuming (13). Inductively coupled plasma spectrometry (I.C.P.S.) is now being applied to a variety of trace element analyses. This newly developed technique is sufficiently sensitive to permit the direct analysis of waters without preconcentration. The ionic emission spectra generated in the high temperature argon plasma substantially decrease the interelement interferences observed in the lower temperature flames of A.A.S. Detection limits for molybdenum in the low µg/L region are being reported by several instrument manufacturers. This instrumentation is very expensive; however, the advantages of high sensitivity, low detection limits, wide dynamic range, and multi-element capability will make this technique very valuable for trace element studies. In Appendices B and C we describe the methods we have used for colorimetric and AAS analyses.

SECTION 5

PRODUCTION AND USE

PRODUCTION

The prinicpal mineral from which molybdenum is obtained is molybdenite (MoS_2) (28). The product obtained from the milling of crude ore containing molybdenum, molybdenite concentrate, generally contains 90% or more MoS_2. Almost all molybdenite concentrate is then converted to technical-grade molybdic oxide (MoO_3) which is the base material for production of various chemical compounds, ferromolybdenum, and purified molybdenum. Some lubricant-grade MoS_2 is prepared directly from the concentrate by additional grinding and flotation.

Technical grade molybdic oxide is usually produced by roasting in a multiple hearth furnace at temperatures up to 600°C (28). Pure molybdic oxide is obtained by sublimation or selective recrystallization of technical-grade molybdic oxide (90-95% MoO_3) at about 1,000°C to 1,100°C. It is used as a base material for metallic molybdenum and for sodium and ammonium molybdates. Technical grade molybdic oxide is added as a charge material or directly to cast iron and to a large proportion of steels and other alloys.

In 1976 production by the United States was 50 million kg (29). The total world production was 76 million kg. Approximately 35% is produced as a byproduct from ores of copper, tungsten, and uranium; the remaining 65% is recovered from ores processed for molybdenum. Approximately 60% of the U.S. production is presently obtained from the Climax and Henderson mines in Colorado which are operated by the Climax Molybdenum Corporation, a division of AMAX, Inc. Other producers include the Molybdenum Corporation of America, Duval Sierrita, and Kennecott Copper Corporation. Two primary and four byproduct producers account for over 95% of the domestic output (30).

Because molybdenum occurs at concentrations of 0.5% or less in ores, substantial amounts of solid waste must be disposed of in tailing piles. In the case of the world's largest mine at Climax, approximately 36,000 metric tons of tailings are generated per day. This operation has released as much as 100,000 kg per year of molybdenum as aqueous effluent.

Figure 2 gives estimated atmospheric release rates for various parts of the production sequence.

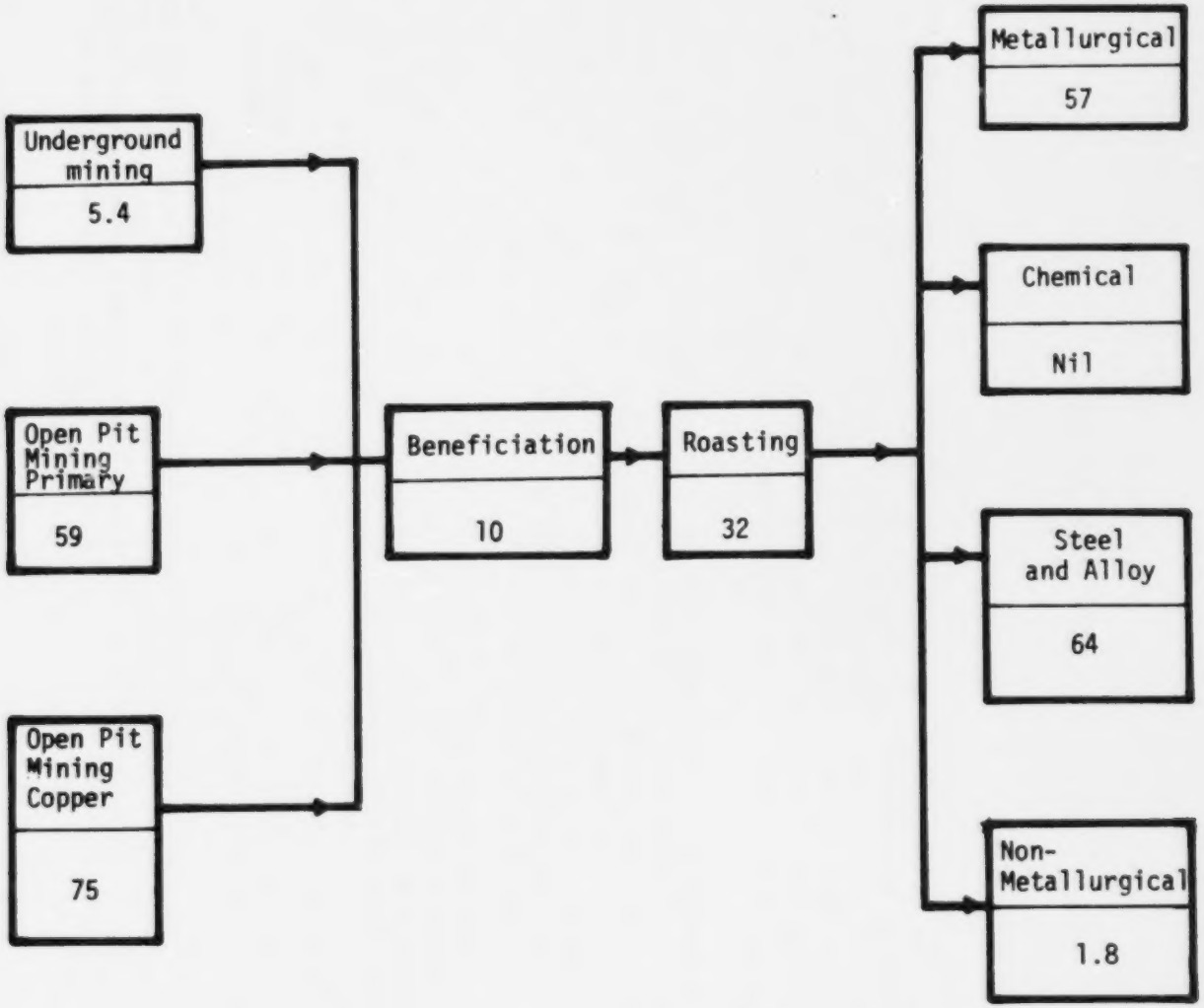

Figure 2. Atmospheric release in metric tons/year molybdenum (31).

INDUSTRIAL USE OF MOLYBDENUM AND ITS COMPOUNDS

The consumption of molybdenum in 1974 is summarized in Table 2. Table 3 lists some common molybdenum compounds and their use.

TABLE 2. CONSUMPTION OF MOLYBDENUM BY END USE (1974) (29)

Alloy steel	44%
Stainless steel	21%
Tool steel	11%
Chemicals and lubricants	8%
Cast iron and steel-mill rolls	6%
Special and super alloys	5%
Molybdenum metal	4%
Miscellaneous	1%

TABLE 3. SOME USE APPLICATIONS FOR MOLYBDENUM AND ITS COMPOUNDS

Molybdenum	iron-base alloys; cracking catalysts; in fertilizers
Molybdic oxide	production of ammonia from hydrogen and nitrogen
Cobalt molybdate	desulfurization of gasolines
Molybdenum disulfide	lubricant
Molybdenum pentachloride	Friedel Crafts chlorination of aromatics and alkylations; vapor phase deposition of molybdenum coatings
Molybdenum hexacarbonyl	vapor phase deposition of molybdenum coatings
Molybdenum acetylacetonate	catalyst for polymerization of polyurethane foam
Molybdenum oxalate	in photochemical systems
Molybdenum dithiocarbamate	lubricant additive
Ammonium molybdate	laboratory reagent for determination of phosphorus, bromates, cholesterol; arsenic in feeds
Molybdenum tannate	coloring of leather
Molybdates (e.g., zinc molybdate)	pigments for printing inks, lacquers, paints; vitreous enamels

One particular use which may increase dramatically over the next few decades is in the catalytic hydrocracking of coal to liquids. If this proves to be feasible on a commercial scale, then to supply 9,490 quadrillion joules (9 quadrillion BTUs) of energy by coal conversion in 1985 we will need to convert almost 363 million metric tons of coal (32). Conversion will require almost 1.8 million kg of molybdenum. While this is a small percentage of the current U.S. production of over 45 million kg/year, one estimate of coal conversion requirements in the year 2000 would imply the need for 11 million kg of molybdenum for this purpose (32). Other energy related uses include the use of molybdenum in elevated temperature steels which are widely used in steam and gas turbines, and in the steam generating sections of fossil or nuclear-fueled power plants. These and other applications are discussed in reference 29.

SECTION 6

ENVIRONMENTAL FATE

ROCKS AND SOILS

The average abundance of molybdenum in the earth's crust is 10^{-4}% or one part per million (ppm) (33). Table 4 summarizes information on the molybdenum contents of some rock types.

TABLE 4. CONCENTRATIONS OF MOLYBDENUM IN VARIOUS ROCK TYPES (33)

Rock type	Range (ppm)	Average (ppm)
Basaltic (igneous)	0.9-7	1.5
Granitic (igneous)	1-6	1.6
Shale	1-3	2
Black shale	1-300	10
Limestone	---	0.4
Phosphorite	5-100	30

Economic molybdenum deposits contain 200 ppm Mo or more, with the lower concentrations generally mined as a byproduct of copper mining. Oil shale from the Green River formation in Colorado contains approximately 30 ppm Mo (34).

The total molybdenum concentration in soils can vary quite widely, but most soils contain between 0.6 and 3.5 ppm Mo (35). The average content of most soils has been reported to be 1 to 2 ppm Mo (36).

Kubota (37) has written perhaps the most recent comprehensive review on molybdenum in soils. His assessment of the data for the United States is that the median for U.S. soils is 1.2 to 1.3 ppm Mo with a range from 0.1 to 40 ppm.

In areas where soil has been developed from a molybdenum-rich formation or where industrial contamination has occurred, the concentrations can be

quite high. Table 5 summarizes data on some soils containing naturally high levels of molybdenum and others which are high because of contamination.

TABLE 5. SOILS WITH ANOMALOUS MOLYBDENUM CONCENTRATIONS

Source	Range	Mean
	ppm	
Natural sources:		
Soils covering a mineralized area (38)	27-190	76
Soil derived from a marine black shale (39)	2-85	12
Alluvial soils - eastern footslopes of Sierra Nevada (37)	5-32	17.4
Soils formed from volcanic ash - Kauai, Hawaii (37)	13.5-18.6	14.9
Industrial sources:		
Soils downstream from a molybdenum mine and mill - Colorado (38)	1.3-4,250	59
Soil irrigated with water contaminated by a uranium mill (40)	49-72	61
Two miles from molybdenum smelter - Pennsylvania (41)	10-72	29

Clearly, both natural and industrial sources can give rise to extremely elevated molybdenum concentrations in soils. Where concentrations exceed 3 to 5 ppm, a geological anomaly or industrial contamination is the likely explanation. Soils adjacent to mineralized areas generally show elevated concentrations of molybdenum and offer a reliable guide to mineralization.

AIR

Concentrations of molybdenum in ambient air are quite low ranging from below detection limits to 0.03 $mgMo/m^3$ (42). Thus, under ordinary conditions there is a negligible contribution from air to human and animal daily intake of molybdenum.

Industrial Exposure - Mining and Milling

In the industrial setting, there can be a considerable exposure to molybdenum. Various environmental levels have been reported in mining operations. In Table 6 levels of dust are reported that were measured during two extensive surveys of a molybdenum mining, crushing, and milling operation.

TABLE 6. COMPARISON OF DUST SURVEY LEVELS IN A COLORADO MOLYBDENUM MINE (43, 44, and Personal Communication, Wenster Area National Laboratory for Occupational Safety and Health, July 1975)

Operation	1959 PHS study total dust impinger method				1975 OCAW study		
	No. of samples*	Range of dust concentration MPPCF†		Avg. dust concentration MPPCF	No. of samples¶	Respirable dust concentration mg/m^3	Avg. respirable dust concentration mg/m^3
Mining	449	71.6 - 0.3	$\%SiO_2$	14.4	45	1.230-0.104	0.471
Crushing	61	98.3 - 0.4	36-39	7.5	11	6.079-0.275	1.318
Milling	33	3.9 - 0.3		1.9	6	0.210-0.046	0.142
Surface shops	35	74.4 - 0.3		4.5	--	---	---
Open pit	--	---		---	14	0.682-0.129	0.318

*Ten minutes stationary samples

†Million of particles per cubic foot - 10 minute samples

¶480 minute personal breathing zone samples

The milling operation is remarkably less dusty than the others due to the high percentage of water used in the process. However, the processes of drying, packing and loading are potential areas of exposure to dust containing high percentages of molybdenite (90% MoS_2).

The data of the 1959 Public Health Service are difficult to use to estimate exposure to molybdenite because the sampling techniques used counted the particles without determining their mass. Therefore, it is not possible to determined the gravimetric concentration of molybdenite in the airborne dust. In our study stationary 10L samples were collected over 10 minute periods. The Oil Chemical and Atomic Workers (OCAW) 1975 study determined the respirable mass of dust at the breathing zone of the worker.* Each sample was collected for 480 minutes (a full shift) and represents about 820 L of sample air. The concentration of molybdenum in the respirable dust collected in the OCAW 1975 study are shown in Table 7. It is possible to estimate the exposure to molybdenum in the mining and processing of molybdenite by assuming that a "standard man" inhales 10 m^3/day during an eight hour workday. This estimation is also found in Table 7.

Industrial Exposure - Smelting

In 1975, the environmental levels of molybdenum were measured in a smelting (roasting) operation (45). Settled dust, impinger samples and high volume, respirable size, filter samples were collected. Concentrations of molybdenum, as MoO_3, in settled dust samples ranged from 57% to 61%. The quantitative analytical method was colorimetry. The molybdenum species were confirmed by x-ray diffraction. Three impinger samples encompassing the roasting operation gave concentrations of MoO_3, which ranged from 3 mg/m^3 to 33 mg/m^3. About 180 L of air were collected and passed through the same sampling trains consisting of two impingers in series. Based on a time-motion study of the exposed workers, the time-weighted average was found to be 9.5 mgMo/m^3.

In order to estimate respirable fraction, three high volume samples were also collected in the same areas where impingers were placed. X-ray diffraction was again used to determine the molybdenum species. MoO_3 was the identified compound and its concentration in the collected dust varied from 52.4% to 77.9% MoO_3. The concentrations of molybdic oxide were then determined by spectrophotometry. Values ranged from 4.6 to 1.1 mgMo/m^3. For an estimation of body burden, see Table 8.

No reports of levels of exposures in industrial operations using molybdenum in the United States were found in the literature.

A few U.S.S.R. studies (46,47) report environmental concentrations of MoO_3. Forty samples were taken above a crucible and in the breathing zone of

*As defined in Interim Guide for Respirable Mass Sampling AIHA Journal, Vol. 31, p. 133, 1970.

TABLE 7. MOLYBDENUM CONTENT OF RESPIRABLE DUST IN A COLORADO MOLYBDENUM MINE
(Estimation of net daily body burden - DBB)
Portable Pump Model G (MSA) with two stage size selective samples
Gelman DM. (MSA PVC 5 µm filters)

Sample number*	Location and classification of worker	Volume of air samples (liters)	Respirable mass concentration µm/m^3 of Mo	Net DBB µg/day†
MINE				
18	Underground loader	795	0.1	---
38	Underground miner	869	0.3	2.2
55	Underground loader	833	3.0	22.5
CRUSHER				
62	Crusher welder	784	49.0	36
66	Crusher mechanic	681	27.9	209
102	Crusher welder	686	8.7	6.5
106	Crusher gyro operator	678	0.1	---
MILLING				
69	Milling mechanic	613	3.9	36.7
68	Milling oiler	787	0.1	0.8
71	Milling mechanic	757	1.5	11.2
98	Milling mechanic	704	12.8	96.0
OPEN PIT				
74	Bull dozer operator	821	1.5	11.2
76	Driller	784	11.7	87.7
93	Loader	819	0.2	1.5

*One blank filter was analyzer for Mo^{6+} for each operation (Mine, Crusher, Milling, Open pit). Concentrations of Mo^{6+} in the blanks ranged from 0.2 to 1.0 µmg. The molybdenum present in the air samples was corrected for the amounts of molybdenum found in the blanks.

†Assuming 10 m^3 of air inhaled during an 8-hour day and 75% absorbed from total respirable dust inhaled.

TABLE 8. MOLYBDENUM LEVELS IN RESPIRABLE DUST AND TOTAL DUST (45)

	Base of roaster	First tier	Second tier
Respirable dust:			
Dust concentration (mg/m^3)	1.31	3.01	6.25
Percent molybdenum in dust	77.8	52.4	71.0
Molybdenum content of dust (mg/m^3)	1.02	1.58	4.49
Total dust:			
$mgMo/m^3$ of air	3.04	9.11	33.28
Hours-exposure/worker*	4	1.5	1.5

$$\text{8-hour TWA} = \frac{(3.04 \times 4) + (9.11 \times 1.5) + (33.28 \times 1.5)}{8} = 9.47\ mgMo/m^3$$

*Does not total 8 hours since there is virtually zero exposure during 0.5 hour lunch break and two 0.25 hour breaks.

workers during a smelting operation involving MoO_3. The concentrations of MoO_3 in the breathing zone of the workers involved in the process averaged 0.22 $mgMo/m^3$. Highest values reported were 0.4 to 0.5 $mgMo/m^3$. The area above the crucible was also sampled showing concentrations from 1.4 to 5.4 $mgMo/m^3$ depending on the molybdenum content of the ore. In another factory producing high purity molybdenum the air concentration ranged from 6.4 to 10 $mgMo/m^3$ of MoO_3. The analytical method was colorimetry but no description of the methods of sample collection is reported (46).

Another U.S.S.R. study reported air concentration in two chemical plants producing molybdenum salts. The range of concentrations found in the first plant was 0.5 to 200 $mgMo/m^3$ of MoO_3. In the second plant the concentrations were 0.2 to 30 $mgMo/m^3$. No analytical methods or sample sizes are given (47).

Clearly industrial exposures can lead to a greatly increased daily intake of molybdenum. Indeed, the present OSHA standard of 5 mg/m^3 soluble molybdenum would, over an eight-hour period, lead to an average intake of 50 mg which is more than 200 times the normal human daily intake.

WATER

It is estimated that 3.6×10^{10} grams of molybdenum are released per year into the surface waters of the world by natural processes (48). Concentrations in most waters are less than 20 $\mu mMo/L$. Average molybdenum

concentrations in sea water range between 4 and 12 µgMo/L (49,50). In 1963, Durum and Haffty (51) estimated the median molybdenum content of major North American rivers to be 0.35 µgMo/L. In an extensive survey of Colorado surface waters, Vogeli and King (52) found that 87% of 299 samples from 197 stations contained less than 10 µgMo/L and concluded that concentrations of more than 5 µgMo/L in the surface waters of Colorado were probably due to molybdenum mineralization and/or molybdenum mining and milling.

Vinogradov (53) found a normal molybdenum background concentration of 3 µgMo/L in the groundwaters of the U.S.S.R. A recent study of groundwaters in Colorado by the U.S. Geological Survey (personal communication) found that 98 out of 156 samples contained less than 1 µgMo/L--only 10 samples contained more than 10 µgMo/L and the highest concentration found was 28 µgMo/L.

Runnells and Kaback (38) compared waters draining highly mineralized areas with waters draining areas containing normal concentrations of molybdenum in rocks and soils. They found that molybdenum mineralization did not contribute significantly to molybdenum concentrations in surface waters. (Most ore bodies contain molybdenum as MoS_2). Water from streams draining highly mineralized areas rarely contained more than 1 to 2 µgMo/L. With the exception of surface waters that are very acidic and have a high content of particulate ferric iron, molybdenum occurs principally in the form of a truly dissolved, filterable species (54).

Normal concentrations in stream sediments, as measured in the -80 mesh fraction, are reported to be in the range of 1 to 5 ppm Mo. Concentrations ranging from 10 to 200 ppm Mo have been found in sediments from streams that drain relatively undisturbed natural deposits of molybdenum in the United States (38). Sediments derived from black marine shales in England may contain as much as 300 ppm Mo (39). The concentration of molybdenum in stream sediments has been shown to increase with decreasing grain size (38). Thus, stream sediments, as opposed to water, do reflect mineralization.

Contamination of surface and ground waters has been documented in several studies. Kopp and Kroner (55) conducted an extensive sampling of water from rivers and lakes of the United States from 1962 to 1967. Water samples were taken from 100 sampling stations in the vicinity of highly populated areas, industrial areas, recreational use areas, state and national boundaries, and other potential problem areas. Of the 100 stations sampled, 38 had maximum concentrations of molybdenum in water greater than 100 µgMo/L and 26 stations had mean molybdenum concentrations in water samples greater than 50 µgMo/L. The detection limit for molybdenum in this study was 40 µgMo/L.

There are many industries involved in the production and use of molybdenum compounds. These industries may be sources of molybdenum contamination of the environment. The only ones that have been studied in detail are molybdenum mining, milling, and smelting; but significant contributions of molybdenum to the environment can result from uranium and copper mining and milling (56), shale oil production (34), and coal-fired power plants (57). The concentrations of molybdenum in the aqueous effluents from these sources can be as high as 850 mgMo/L (a uranium mill) (58), and the release rates can be as high as 100,000 kg of molybdenum per year (a molybdenum mill) (58).

Table 9 shows data for some waters receiving various industrial effluents. A more detailed discussion of industrial sources is contained in another part of this section, Industrial Sources.

TABLE 9. MOLYBDENUM CONTENT OF WATERS AND STREAM SEDIMENTS CONTAMINATED BY VARIOUS INDUSTRIAL ACTIVITIES

	Water		Stream Sediment	
	Mean	Range	Mean	Range
	----- µg/L ----		----- ppm -----	
Stream below molybdenum mine and mill (Colo.-1973)	---	100-10,000	530	50-1,800
Stream below molybdenum tailings pile (N. Mex.)	600	---	21	---
Ground water down gradient from uranium mill (Colo.)	50,000	---	---	---
Coal-fired power plant ash pond effluent (N. Mex.)	170	---	---	---
Leachate from retorted oil shale (Colo.)	4,100	2,500-8,300	---	---

A survey of the finished water supplies of the 100 largest U.S. cities by Durfor and Becker in 1964 (59) found a median concentration of 1.4 µgMo/L, with a maximum value of 68 µgMo/L. Hadjimarkos (60) reported that the mean concentration of molybdenum from 161 sources of finished water in 44 states of the United States was 8 µgMo/L.

In an epidemiologic study of the relationships between water constituents and cardiovascular disease, municipal drinking water supplies were sampled in 35 geographic areas of the United States (61). Thirty-three percent of the 3,676 samples tested contained detectable (greater than 1 µgMo/L) amounts of molybdenum. The highest mean concentration found for an area was 52 µgMo/L. Only those samples with detectable amounts of molybdenum were included in the computation of area means. The maximum concentration in any sample, 276 µg Mo/L, was in a sample of tap water collected in Denver, Colorado in 1975 (62). Some samples collected in Ohio, West Virginia, and South Carolina had maximum molybdenum concentrations greater than 90 µgMo/L. Some Nebraska, Kansas, Florida, Washington, Delaware, Tennessee, and California samples contained between 20 and 90 µgMo/L maximum molybdenum concentrations.

Tsongas carried out an extensive survey of tap waters in the Denver metropolitan area and in several Colorado mountain communities for our study and for a previous investigation in 1974-1976 (63). Community tap waters which were impacted by molybdenum mining and milling operations contained molybdenum at concentrations between 1 and 500 µgMo/L. Golden, Colorado derives its

water supply from a stream draining a molybdenum mine and mill site, and during 1971, tap water samples contained an average of 440 µgMo/L. During late 1974, molybdenum concentrations were still averaging 440 µgMo/L, when the mill ceased operation. However, by January of 1975, the tap water molybdenum concentrations had decreased to about 150 µgMo/L. In June 1975 the concentration was found to be 60 µgMo/L. The decrease was probably due to the spring runoff. The concentration of molybdenum in tap water samples from Golden during 1977 had a mean of 30 µgMo/L.

Another suburban Denver community receives water from the same source, but stores the water in a large reservoir prior to finishing and distribution. The molybdenum concentration of this community's domestic water has decreased at a slower rate than that of Golden, to approximately 80 µgMo/L by October 1977.

Frisco, Colorado, a mountain community, derives its domestic water from Ten Mile Creek, which drains another large molybdenum mining area. Silverthorne, Colorado receives its water from the Blue River just below Dillon Reservoir. Tap water molybdenum concentrations in these two communities have varied between 100 and 400 µgMo/L from 1974 to 1977.

Denver, Colorado draws water for domestic use from Dillon Reservoir at certain times of the year. The concentration of molybdenum in Denver's tap water varies considerably, depending upon tap location and time of year. Since 1975 the molybdenum concentrations in tap waters in Denver have varied between less than 1 µgMo/L and 80 µgMo/L. Barnett and others (64) reported molybdenum concentrations in some Denver tap waters as high as 190 µgMo/L in 1969. Greathouse and others (62) report a maximum value for tap waters sampled in the United States of 276 µgMo/L from a Denver tap in 1975.

It can be concluded that concentrations of more than 20 µgMo/L in surface, ground, or tap waters are very likely to be anthropogenic.

PLANTS

Molybdenum plays an important role as a micronutrient for plants. In 1942 molybdenum was discovered to be a limiting factor to clover production in some Australian pastures (65). Microorganisms require molybdenum for nitrogen fixation and for the enzymes which catalyze the reduction of nitrate to nitrite. Molybdenum is now a common component of fertilizers in many parts of the United States. Several soil parameters can influence the availability of molybdenum to plants. These include soil pH, soil moisture, sulfate, and phosphate. Molybdenum availability is generally higher at high pH, low sulfate, high moisture levels, and high phosphate (37,65).

While normal concentrations of molybdenum in plants are 1 to 2 ppm, a range of tenths of a ppm to hundreds of ppm have been observed (65). Legumes appear to take up more molybdenum than other plants. In 1938 Ferguson and co-workers (66) reported that a severe disease in cattle was cause by abnormally high concentrations of molybdenum in forage. When the molybdenum in forage exceeds 5 ppm it can adversely affect the health of grazing cattle (39,67).

The additions of amounts of molybdenum ranging from a few hundredths of a kilogram per acre per year to several kilograms per acre per year (depending on various soil parameters, climate, etc.) can significantly increase the molybdenum content of plants (68). Irrigation with water containing anomalous concentrations of molybdenum can lead to plant uptakes that could be deleterious to animal or human health if these constituted an important part of the diet (69). The Water Quality Criteria Committee (70) recommended a maximum concentration of 10 µgMo/L in irrigation water for continuous use on all soils.

Vlek (71) and Lindsay developed a model for molybdenum uptake that predicts molybdenum concentrations in plants as a function of molybdenum in irrigation water and other parameters. For a common Colorado soil they found that the use of irrigation water containing 100 µgMo/L would lead to plant concentrations toxic to cattle in approximately 15 years.

FOOD

The molybdenum content of food varies with the type of food and the geochemical region in which it is grown. Legumes, cereal grains, leafy vegetables, liver, and kidney beans are among the foods which usually contain higher concentrations of molybdenum than fruits, root and stem vegetables, muscle meats, and dairy products (72).

Deosthale and Gopalan (1) found widely differing molybdenum contents (0.21 and 1.39 µgMo/gm) in two varieties of Sorghum vulgare Pers. grown and used as an important dietary staple in some regions of India. Indian rice was reported to contain about half as much molybdenum as the sorghum (73).

Other investigators have found great variability in the molybdenum content of foods (74,75). The variation in content was as great in samples of the same food from within a region as it was between samples of the same type of food grown in different areas of the world (75).

Tsongas and co-workers (76) in our study determined the molybdenum content of foods collected in a market basket sampling program which sampled foods from six major supermarket chain stores in the Denver, Colorado metropolitan area. There was very little variation found in the molybdenum content of particular food items from store to store, and no seasonal trends in the molybdenum content were apparent in samples collected over one-and-a-half years.

In our study (77), 10 food items making up the greatest bulk in the 'typical American' diet were sampled most extensively. Of these, 'white' enriched bread and eggs contained the highest concentrations of molybdenum on a wet weight basis. Ground beef, iceburg lettuce, and apples were lowest in molybdenum. Analysis of supplemental samples provided data on the molybdenum concentration of foods in each of 24 U.S.D.A. food categories (78) (see Table 10).

Data on the average molybdenum concentration of milk samples (whole, skim, and 2% butterfat) collected during this study are consistent with those

TABLE 10. MOLYBDENUM CONTENT OF FOODSTUFFS

	Mean Mo µg/gm wet wt.
Milk, milk drinks	0.06
Cream, ice cream	0.06
Cheese	0.11
Eggs	0.086
Beef	0.04
Pork	0.029
Meat mixtures	0.039
Poultry: turkey, chicken	0.047
Fish	$\leq$ 0.01
Legumes: dry beans, peas, lentils, mixtures	1.63
Nuts, nut butter	0.02
Fats, oils	$\leq$ 0.01
Breads	0.21
Bakery	0.27
Cereals: cooked and ready-to-eat	0.55
Mixtures: pastas, spaghetti, macaroni	0.41
Tomatoes	0.039
Citrus fruits	$\leq$ 0.01
Other fruits, fruit mixtures	0.036
Dark green vegetables	0.03
Deep yellow vegetables	0.24
Potatoes	0.065
Other vegetables, mixtures	0.15
Sugar, sweets	$\leq$ 0.01

previously reported from Colorado (63,78), California (79), the United Kingdom (80), Germany (81), and other areas (82). Average values for milk sampled in all of these areas varied between 20 and 60 µgMo/L. Several investigators have found that changes in the dietary molybdenum intake of cows do affect the molybdenum content of milk (82,83), and the concentrations can vary quite considerably on a local level. However, when compared with other foodstuffs, milk does not usually contain high concentrations of molybdenum. It is important to note, however, that the greatest portion of the dietary molybdenum

intake among children in the United States is provided by milk (76). Milk is consumed in greatly varying amounts by different segments of the population of the United States. It is therefore important to consider the trace element content of foods relative to their contribution to the total dietary intake when examining the impact of trace elements in the diet. Further information on the daily intake of molybdenum can be found in Section 8 under Biological Effects of Molybdenum in Humans.

INDUSTRIAL SOURCES

Coal Combustion

Coal combustion is probably the largest source of molybdenum to the atmosphere. It has been estimated that 550 metric tons of molybdenum were emitted due to coal combustion in the United States in 1970, as compared to 900 metric tons of molybdenum emitted from all air pollution sources (80). The molybdenum concentration in coal varies widely with the concentrations in western coal ranging from 1 to 15 ppm (57). Mass balance studies indicate a successively increasing enrichment in the outlet ashes. A single 1,000 megawatt power plant may emit 909 metric tons of molybdenum per year (57).

Kaakinen (57) investigated the molybdenum concentrations and bioavailability in bottom ash and fly ash. He found concentrations ranging from 3 ppm in the bottom ash to 37 ppm in the fly ash collected by the electrostatic precipitator. Approximately 15% of the molybdenum in the bottom and fly ash is available for uptake by plants. Dreesen and others (84) found that approximately 60% of the molybdenum in the coal ash from one plant in New Mexico could be extracted from the ash obtained from the ash pond at this plant.

Thus, as the use of coal increases, especially with the impetus to change to coal from other fuels, the emission, both aqueous and atmospheric, of molybdenum from this source will become increasingly important. Since the release into the surface waters of the United States averages about 1,800 metric tons per year (85), the atmospheric emissions alone due to coal combustion were approximately one-third the total background value in 1970.

Other energy sources such as oil shale (34) and uranium (58) are also sources of molybdenum contamination. Some specific instances involving uranium mining and milling are discussed in the part on Uranium Mining and Milling.

Molybdenum Mining and Milling

Three molybdenum mining and milling operations have been investigated in detail: the Climax and Urad operations in Colorado and the Questa operation in New Mexico (38,58). While these facilities dealt with the mining and concentration of molybdenum as MoS_2, which is very insoluble, sufficient solubilization occurred during the operations to lead to concentrations on the order of 1,000 to 10,000 µgMo/L in the waters that are decanted from the tailings. The quantities of water released to surface streams depended on the size of the operations, on the amount of water which was recycled, and on the quantity of precipitation and run-off into the tailings ponds.

The largest releases which were seen were those from the Climax mine in Colorado. Because of large quantities of water from snow melt, the operation had to release 3.7 to 6.2 billion liters per year (3,000 to 5,000 acre feet per year) of water from the tailings ponds into the receiving stream. The total molybdenum release was about 100,000 kg in 1972 (86). The result was a significant increase in the molybdenum levels in surface waters downstream. In 1972, Dillon Reservoir, which is about 10 miles downstream from Climax, had an average molybdenum concentration of about 300 µgMo/L (86). Climax is now taking steps to reduce its release to about 6,800 kg per year by diverting run-off water around the tailings ponds and treating any water that must be released.

The Urad operation (which ceased production in 1974) and the Questa operation are much smaller than the Climax operation and have much smaller release rates. Concentrations of molybdenum in receiving waters for these operations vary from 560 µgMo/L to 1,500 mgMo/L. Since the receiving stream for the New Mexico operation flows into the Rio Grande and is greatly diluted before any significant human use occurs, the only concern with this effluent is that of potential impacts on livestock through irrigation. The release from the Urad mine did lead to very high concentrations (400 to 500 µgMo/L) in the tap water communities downstream. The concentration of molybdenum in these water supplies has decreased substantially since the operation ceased.

Molybdenum Smelting

The most thoroughly documented study of environmental contamination due to molybdenum smelting was reported by Hornick and others (41). They investigated an area surrounding a plant located in western Pennsylvania. Stack emissions, which were reported at 25 to 100 kg per day, had led to increased molybdenum concentrations in soils and plants in the area surrounding the plant. Soil concentrations in nearby farms were as high as 72 ppm Mo and molybdenosis was reported in some cattle. In addition, one sample of water from a nearby stream was found to contain 1,000 µgMo/L. Near a molybdenum smelting plant in the Netherlands forage was found to contain molybdenum concentrations as high as 80 ppm (87).

Uranium Mining and Milling

Molybdenum is frequently found in high concentrations in uranium ores. As a result it is frequently a major contaminant in the effluents from uranium operations. Three cases of substantial molybdenum contamination of the environment associated with uranium mining and milling have been reported. These involved a uranium mill in North Dakota (88), a uranium open pit mine in Texas (89), and a uranium mill in Colorado (58).

The North Dakota operation involved a plant where a uraniferous lignite coal was ashed to upgrade the uranium content. Fly ash released from the plant gave rise to increased soil and forage levels of molybdenum in a nearby farm and a severe problem of molybdenosis in cattle.

The Texas case involved open pit mines which intercepted shallow aquifers. Elevated molybdenum concentrations in surface waters, soils, and forage were reported and molybdenosis in livestock was documented.

The Colorado mill began operation in 1953. In 1965 farmers located down hydrologic gradient from the mill reported a deterioration in the health of their cattle. A study of the mill and the surrounding area indicated that leakage from the tailings ponds containing 860,000 µgMo/L contaminated the underlying aquifer. The water from the farms' wells used for irrigation and stock water contained as much as 50,000 µgMo/L. Forage taken from the farms was shown to contain as much as 300 ppm Mo.

Aqueous effluent from some uranium operations in New Mexico are reported to contain as much as 1,000 µgMo/L (personal communication). Uranium mining and milling, therefore, can contribute significant amounts of molybdenum into the environment, particularly as an aqueous effluent which can affect drinking water quality.

Steel and Copper Milling, Oil Refining, and Claypit Mining

While the largest use of molybdenum products is in steel, very little is known about emissions from steel plants. One study reports highly elevated concentrations of molybdenum in forage accompanied by molybdenosis in cattle downwind from a steel plant (90).

In some copper milling operations, molybdenum is the major contaminant in the aqueous effluent. Concentrations in the effluent have been measured to be as high as 30,000 µgMo/L (91).

Since molybdenum-containing catalysts are used in the refining of oil, oil refineries represent another possible source of molybdenum into the environment. One such incident involved a refinery in England which was reported to have lost 0.45 to 3.6 metric tons of molybdenum per month during 1960 and 1961 (92,93). Elevated molybdenum concentrations in forage, due to these emissions, resulted in molybdenosis of cattle.

In Missouri a claypit was found to be the source of molybdenum which caused severe problems in a nearby herd of cattle. Some plants in the area were found to contain as much as 750 ppm Mo (93).

REMOVAL TECHNOLOGY

The most thorough study of the removal of molybdenum by conventional water and wastewater treatment was reported by Zemansky (94). In this study twelve water treatment plants, ten wastewater treatment plants, and two wastewater tertiary treatment pilot plants were sampled at various times over a one year period. The samples were analyzed for 19 trace metals including molybdenum. The removal of molybdenum in conventional plants was very low, averaging only 15%. The plants studied included the Alvarado Plant in California which supplies part of the water for San Diego as well as treatment plants in Boulder, Denver, Golden, and other communities in Colorado. The plant which had the highest molybdenum concentrations was the Golden, Colorado plant. At that time the average concentration during the year was 360 µgMo/L in the influent. The average removal for the plant was 14%.

Zemansky concluded that the reason for the consistently low removals of molybdenum was the fact that molybdenum is present primarily as the molybdate anion. Since many colloids present in water are negatively charged, sorption and removal of molybdenum does not occur, except perhaps where an excess of alum is added (a situation which is generally avoided).

Zemansky also found that most wastewater treatment plants have a very low percentage removal of molybdenum. For most plants only 4% to 16% removal was observed. Carbon adsorption was observed to be moderately effective (about 50% removal) at the Lake Tahoe plant.

Runnells and others (95) showed that significant quantities of molybdenum were removed from solution below an outfall of acid mine drainage. They concluded that the presence of ferric iron and the low pH led to the insolubilization of molybdenum. Zander (96) demonstrated that this technique could be used to remove molybdenum from industrial waste streams. The process used involved the addition of ferric iron and subsequent dissolved-air flotation. Removal efficiencies of better than 99% were obtained. Typical molybdenum concentration in a treated effluent which initially contained 15,000 µgMo/L was 110 µgMo/L.

One molybdenum mining and milling operation is using ion exchange to treat their effluent. They report removal efficiencies of approximately 98% in treating water containing 6,000 µgMo/L (personal communication).

SECTION 7

BIOCHEMISTRY AND METABOLISM

BIOCHEMICAL FUNCTION

The biology of molybdenum is a vast topic which is described in the scientific literature in several languages. No attempt will be made here to give an exhaustive treatment of the subject, especially as several excellent recent reviews are available (50,82,97). Utilizing these sources a brief outline of the biological function of molybdenum is presented with special reference to our approach to molybdenum-related effects in bone, gut, and blood cells.

Molybdenum is rapidly absorbed from food and water by gut and placenta (98) when present as the moblydate or trioxide, but not as the disulfide, and it is rapidly excreted via the urine in man (99). Exogenous or endogenous inorganic sulfate specifically increases molybdenum excretion and reduces its retention in tissues (100). Molybdenum also affects the utilization of copper--increased molybdenum intake resulting in copper depletion and vice versa (101). The protective action of copper and sulfate on molybdenum toxicity has been utilized in the therapeutic administration of copper sulfate to livestock suffering from molybdenosis (102). Tungstate is a molybdenum antagonist and can induce a functional molybdenum deficiency (103). Molybdenum may mediate the release of iron from intestinal mucosa (104) but the importance of this effect is being questioned. Molybdenum is widely distributed in the tissues of the body ranging from approximately 3 ppm Mo in liver to approximately 0.15 ppm Mo in lung, brain, and muscle (82). The skeleton contains over 50% of the total body molybdenum, presumably due to the large surface area of the mineral phase of bone and the possibility of phosphate-molybdate exchange reactions in hydroxyapatite crystals. Bone, tendon, and cartilage abnormalities, as well as osteoporosis (105), have been seen in animals with molybdenosis. Tooth enamel has appreciable quantities of molybdenum of the order of 5 ppm and was thought to confer a degree of caries-resistance, but this has been disputed, and the effects seen are thought to be due to an effect of fluoride alone (106).

Molybdenum in blood is present in the plasma and is also firmly bound to red cells (107), values of less than 5 to 15 ppb being common in the general population. The homeostatic control of molybdenum is not as efficient as that for sodium and potassium since molybdenum levels several times the normal range are observed in industrial workers exposed to molybdenum (108).

The essential biochemical function of molybdenum is related to the activities of the enzymes xanthine oxidase (109), aldehyde oxidase, sulfite oxidase, nitrogenase, and nitrate reductase (82). Molybdenum participates in the

reaction of xanthine oxidase with cytochrome C and facilitates the reduction of cytochrome C by aldehyde oxidase (110). In milk, xanthine oxidase has two molecules of FAD, 2g. atoms molybdenum, and 8g. atoms Fe/molecule of protein. Three isozymes of xanthine oxidase are present in milk. One of these enzymes (KO_2) contained only molybdenum whereas the other two (KO_4a and KO_4b) contained copper (111). Adaptation to feeding molybdenum or copper occurred by changing the metal content of these isozymes. Xanthine oxidase in the body converts hypoxanthine to xanthine and xanthine to uric acid. High intakes of molybdenum in man (10 to 15 mgMo/day) have been associated with an increased incidence of uric acid gout. Lower intakes, up to 1.5 mgMo/day, had no observable effect on uric acid excretion but increased copper excretion (108,111).

In order to study the basic chemistry of molybdenum, nonenzymic models for the nitrogenase system have been proposed utilizing complexes of molybdate thioglycerol or cystine and Na BH_4 acting on substrates of nitrogenase including nitrogen (112). The rates of substrate reduction in the model system were lower but parallel to those of nitrogenase. Nitrogenase reductions, although exothermic, were accelerated by ATP to a greater extent than by ADP and AMP. To explain this effect it was suggested that nucleotides form protonated complexes with Mo^{ox} which catalyzes the hydrolysis of the nucleotide phosphate. Mo^{ox} is converted to Mo^{red} through a dehydroxylated derivative of Mo^{ox}. Thus, molybdenum in the complex is activated by ATP for its role in nitrogenase-like behavior. The efficiency of this model complex could be enhanced by the addition of iron in the form of ferredoxin model compounds.

Work of the Colorado Molybdenum Project

The investigators of the present project were faced with the difficult problem of measuring molybdenum effects at low levels of exposure. Consequently, in order to detect the biochemical effects of molybdenum, very sensitive subclinical tests were devised in the area of bone metabolism, xanthine oxidase in red blood cells, and adenosine triphosphate (ATP) metabolism in erythrocytes and platelets.

Bone Calcium Transport--

This model system employs the excised ulna of the rat with periosteum intact and measures the *in vitro* transport of calcium 45 from bone to a physiological bathing solution pumped over the bone (113). The rate constants computed for the mathematical model of this three compartmental system indicated a dose response for rats supplemented with molybdenum at the levels of 10 mgMo/L and 100 mgMo/L in their drinking water (114). The most pronounced effect on calcium transport related to molybdenum exposure was a significantly increased loss of exchangeable calcium from the bone at a rate approximately three times the normal rate at a concentration of 100 mgMo/L in the water. Bone molybdenum content increased almost eight-fold from 0.71 ppm to 5.3 ppm of ash. No significant changes were seen in bone calcium and phosphate contents.

The model enables one to study the effects of molybdenum ingestion in animals at relatively non-toxic levels in water or food but would not be suitable for lower concentrations--lower than 1 mgMo/L. Application to humans would also be difficult to standardize and use due to the necessity for a bone

biopsy. Nevertheless, this technique is useful in defining the site of skeletal action of molybdenum, and the results were consistent with the osteoporosis associated with molybdenosis in animals (82).

Human Blood Cell Biochemistry--

The screening tests for humans included: (a) xanthine oxidase activity of erythrocytes using xanthine and hypoxanthine as substrates, (b) ATP production by red cells and platelets from C^{14} adenine, and (c) total intracellular adenine nucleotide pools. Xanthine oxidase activity, producing uric acid, ATP production from C^{14} adenine, and the total cellular pools of adenine nucleotides and related purines were determined by a combination of thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) (115). Several groups of people were studied ranging in exposure to molybdenum from workers at a Denver molybdenum processing plant to surrounding communities on much lower average exposure to molybdenum. These methods are being prepared for publication.

C^{14} Uric Acid Production--

The results of C^{14} uric acid produced from either xanthine or hypoxanthine are reported in Table 11.

TABLE 11. URIC ACID PRODUCTION BY LYSED HUMAN ERYTHROCYTES USING THIN LAYER CHROMATOGRAPHY

Location	Uric acid cpm/mL range x 10^{-3}	Serum uric acid mg% range	Serum Mo µg/L range	Substrate 105cpm	µg Mo ingested/day*	N
Mo Denver processing plant	1.5-5.0 2.1-4.4	4.4-7.9	18-363	Xanthine Hypoxanthine	10,000-50,000	15
Summit County†	0.1-2.2 0.1-2.0	3.6-8.4	5-33	Xanthine Hypoxanthine	400-500	23
Marsden	1.6-2.7 1.5-8.0	3.1-9.4	5-13	Xanthine Hypoxanthine	200-300	14
Denver suburb	0.1-4.9 4.0-6.0	5.0-9.0	5-25	Xanthine Hypoxanthine	300-400	10
Golden	3.0-5.0 5.5-8.0	4.1-7.1	5-43	Xanthine Hypoxanthine	500-600	8

*Estimated from total intake of food, water, and air.

†One individual with gout who had a uric acid production of 76×10^3 cpm/mL RBC hemolysate was excluded from the range. This was confirmed by HPLC detection and identification of uric acid.

There was a considerable degree of scatter and no significant correlation between C^{14} uric acid production and serum molybdenum or uric acid concentration was found within any group.

C^{14} ATP Metabolism--

Table 12 shows the results of C^{14} ATP formation and related substances from C^{14} adenine precursor by red cells and platelet-rich-plasma (PRP).

TABLE 12. RESULTS OF C^{14} ATP FORMATION

Location	$C^{14}R = \frac{ATP+ADP}{AMP}$	Cells	Serum Mo: µgMo/L
Denver Mo plant*	R	RBC	18-363
	1.5-7.8	PRP	18-363
Summit County	2-40	RBC	
	4-34	PRP	5-33
Marsden	Not viable	RBC	5-13
	Not viable	PRP	
Denver suburb	2.5-11	RBC	
	4.9-14	PRP	5-25
Golden	6.8-12.2	RBC	
	2.0-17	PRP	5-43

* Only 31% of this group had R values in the normal range, whereas over 90% of all the other groups had normal R values, i.e., 4.5 or higher. In this group alone a negative correlation of R with serum uric acid concentration of r = -0.84 was seen (Fig. 3). A plot of R versus log (serum Mo) is shown in Figure 4. The correlation coefficient here is also -0.84.

When sodium molybdate was added *in vitro* to platelets or red cells at a concentration of 0 to 100 ppm no significant change in ATP production or pool size was seen.

Discussion

Finding methods of suitable sensitivity and capable of adaptation to field studies on humans was difficult. Nevertheless, by the end of the period of support considerable skill had been developed in analyzing blood from volunteers in batches of 20 per day. Xanthine oxidase activity in hemolyzed red cells is low compared to other tissues such as liver or intestine, but is measurable using the isotopic technique. One subject with clinical gout had greatly increased levels of red cell uric acid production. However, no significant correlation of xanthine oxidase with serum molybdenum was found.

With regard to platelet ATP metabolism, the molybdenum smelter workers had disturbances which were significantly correlated with both serum molybdenum concentration and uric acid concentration which was elevated in this group. None of the other groups had significant deviations of platelet metabolism. Whether these abnormalities were solely due to the effect of molybdenum or whether other factors in the working environment contributed to the

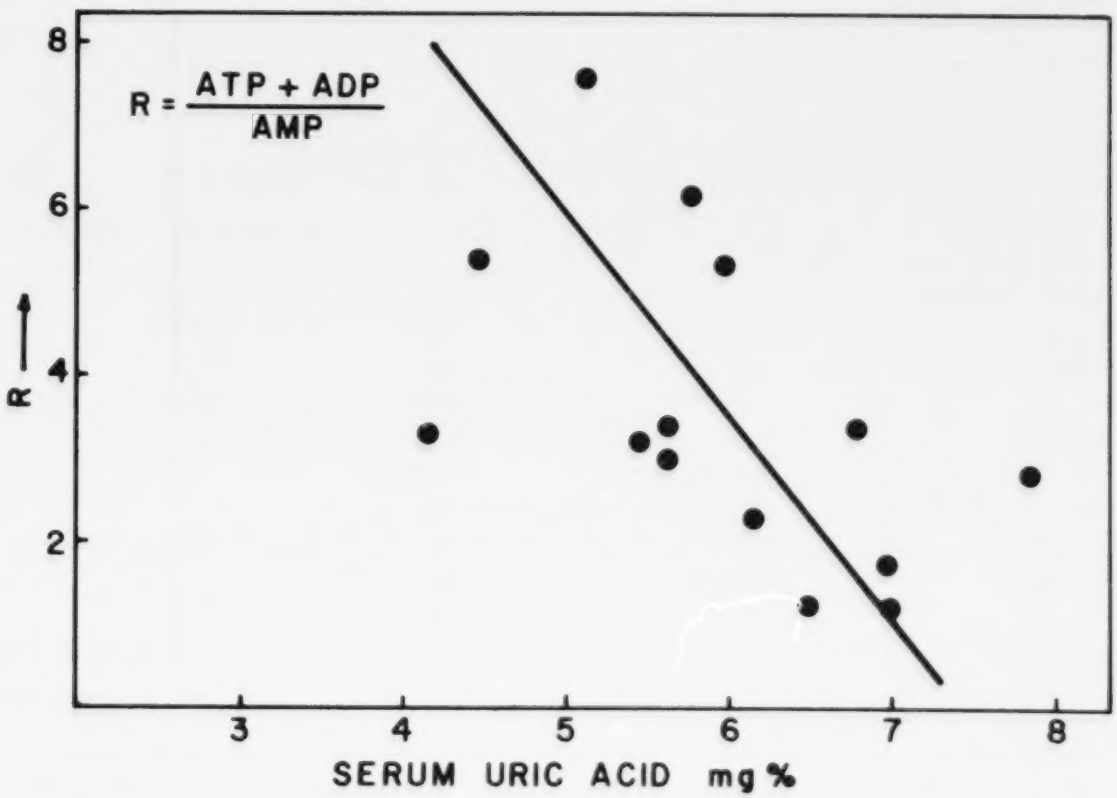

Figure 3. R value versus serum uric acid concentration (r = -0.84).

results could not be determined. However, if plans to reduce the exposure to molybdenum-containing dust are carried out it should be possible to re-study these individuals after their blood levels of molybdenum have decreased to normal.

Conclusions

Sensitive methods for detecting potential environmental toxicity have been devised.

Significant changes in platelet adenine metabolism were seen in industrial workers processing MoO_3, but not in any of the non-industrial populations on various molybdenum intakes.

No changes in red cell xanthine oxidase activity were seen in any of the subjects which could be related to exposure to molybdenum.

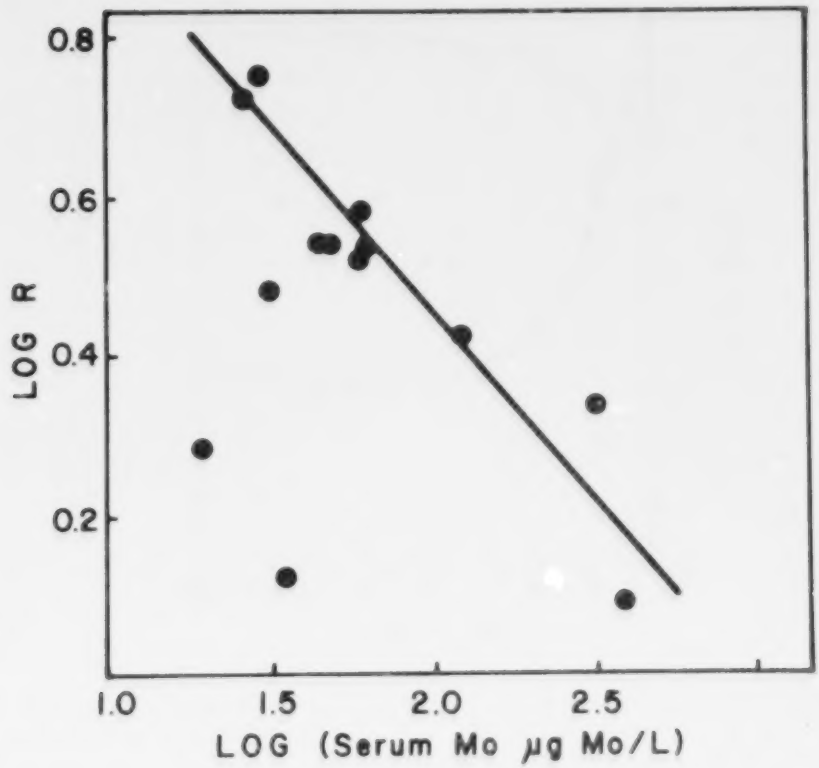

Figure 4. R value versus log (serum Mo) (r = -0.84).

METABOLISM

For trace elements, little is known about the bioavailability, absorption, transport, elimination, or movement in and out of tissues (116). Data on these aspects of the physiology of molybdenum are mostly limited to the soluble inorganic forms such as sodium molybdate (Na_2MoO_4) (82) and molybdic trioxide (MoO_3) (117,118). Balance and tracer studies have shown that these forms are readily absorbed in the gut of non-ruminant animals such as rats (118-122) and swine (98,101,123-125) and that most intake is eliminated in the urine within 24 hours (Fig. 5). In ruminants, elimination of molybdate is much slower. Six to seven days are required for elimination of most of a dose and only 10% to 15% of the dose appears in the urine. The remaining fraction appears in the feces (116). While most researchers have studied relatively soluble molybdenum compounds, Fairhall and others (117) also exposed guinea pigs to dusts of molybdenite (MoS_2), a highly insoluble compound. The tissue concentrations, with the exception of the lungs, of the exposed animals did not differ significantly from the controls; whereas exposures to dusts containing similar concentrations of molybdic trioxide and calcium molybdate led to greatly increased molybdenum concentrations in the liver, kidney, and other

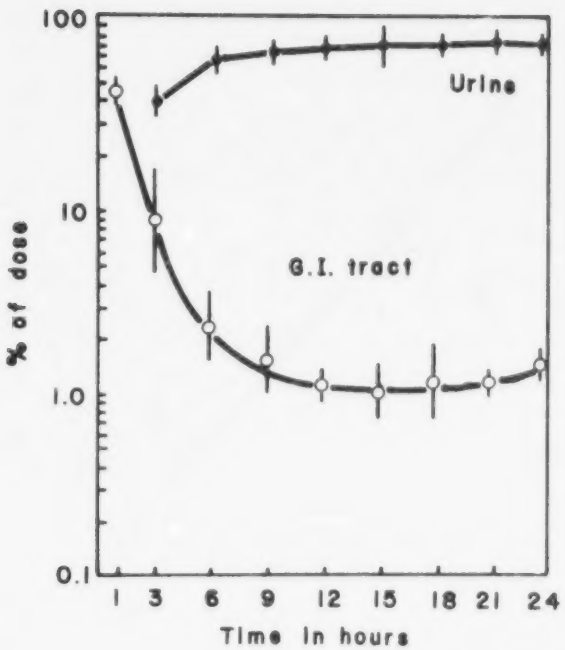

Figure 5. Percent of molybdenum-99 dose remaining in the gastrointestinal tract versus time, and percent of dose in accumulated urine versus time after dosing.

tissues. Since the principal form of molybdenum found in natural waters is molybdate (see Section 3), animal absorption studies using this chemical species are useful for determining a drinking water criterion.

Pharmacokinetic studies of sodium molybdate (Na_2MoO_4) were performed in this laboratory (119,126,127) using starved rats. The animals were force-fed doses of molybdenum-99 as the molybdate salt. Eighty-three percent of the dose was absorbed in the stomach, 13% in the upper small intestine, and 3% in the lower small intestine. No absorption occurred in the large intestine. These results agree with the less extensive work of Bibr and Lener (120), and Nielands and coworkers (118). An absorption half-time of 0.88 hours was measured for both the stomach and the upper portion of the small intestine. The similarity of absorption rates may indicate that the absorption mechanism is the same in both parts of the gut. Uptake through the stomach wall was also observed by others (120). Our data on the stomach are consistent with passive movement through intercellular pore spaces.

It may be, however, that absorption in the intestine can proceed by a different mechanism. Richert and Westerfeld (128) suggested that xanthine oxidase might be a carrier for the transport of molybdenum. They based their

suggestion on data which showed that intestinal xanthine oxidase activity was correlated with ingested molybdate. We have successfully repeated this work (129). In addition, we found that xanthine oxidase activity was highest in the duodenum, the first part of the small intestine, and lower in the ileal segment. Cardin and Mason (130) performed *in vitro* studies which showed that molybdenum absorption occurred throughout the small intestine. The highest rate was observed in the ileum, the last portion of the organ. Since molybdate absorption is highest at the opposite end of the intestine from the region of highest xanthine oxidase activity, it may be that the function of the enzyme in the intestine is not as of a carrier for molybdate.

Sulfite oxidase activity, on the other hand, has been shown to be highest in the ileal segment (131) but there is no reason to assume that it is acting as a carrier. The lack of a clear interpretation of all of these studies indicates that much remains to be learned about the complex nature of molybdenum absorption.

Additional evidence for the high level of absorption of molybdate has been provided by balance studies both here and in other laboratories (98,101, 118,120,121-125). These studies have shown that between 1% and 27% of the intake of molybdenum appears in the feces with the remainder in the urine. This indicates that most of the molybdenum is absorbed. Some of the inconsistencies between laboratories and the variability of results may be due to differences in the amounts of solid food in the diet. Balance studies performed in this laboratory have shown that larger percentages of molybdenum appeared in the feces when the animals obtained molybdate mixed into their food (10% to 15%) than when it was dissolved in their drinking water (5% to 7%). This finding indicates the importance of performing additional studies in which different chemical species of molybdenum are supplied to the animals in different matrices.

Our pharmacokinetic studies have shown that after molybdenum-99, given as sodium molybdate, is absorbed in the gut, it moves into and out of the blood very rapidly. No more than 0.2% of the dose was measured in the blood at any time (126). The kinetic profiles of movement into and out of most tissues were similar to the profiles observed for blood. These profiles were characterized by a maximum accumulation at three to six hours and then an initially rapid elimination followed by a much slower loss (see Fig. 6). The liver, thyroid, and adrenals showed different profiles. Accumulation in the liver reached a maximum (1.5% of the dose) in the first hour (earlier than most other tissues) (Fig. 7). After six hours it had declined to 0.7%, a level which was maintained essentially to the end of the test. At the end of the test the liver retained more of the dose than any other tissue. This behavior is consistent with the fact that the liver has been found to have the highest concentration of molybdenum of any tissues in both man (132) and other non-ruminants (116).

Both the thyroid (127) and the adrenal glands (126) showed secondary uptake of the molybdenum-99 (Fig. 7). In both glands there was the typical, initially rapid loss followed by a slower loss up to 15 to 18 hours. At this time the decrease in accumulated molybdenum was reversed and it then slowly increased through the remainder of the 24-hour test period. The explanation

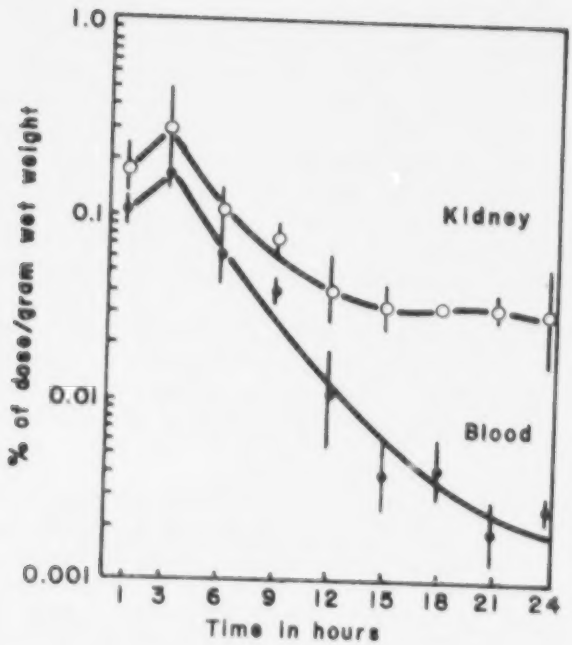

Figure 6. Percent of molybdenum-99 dose accumulated per gram of tissue (wet weight) for kidney and blood versus time after dosing.

of these results is not clear but they agree with those from other laboratories (132) which showed relatively high levels of molybdenum in both these glands. Such accumulation, when dietary intake of molybdenum is elevated, might have detrimental effects on the stress response in which the secretions of both these glands play an important role. Results of experiments which indicate such effects are discussed in Section 8.

Elimination of molybdenum in the urine was very rapid. The kinetics are biphasic and they closely follow the changes in the blood (Fig. 5). Seventy percent of a dose is eliminated in the first three to six hours. The rate of elimination then decreases and 87% of the dose has been eliminated after 24 hours. These percentages are consistent with the results of Bibr and Lener (120), but they are much higher than those of other researchers (e.g., 118). It can be seen that molybdenum is eliminated rapidly in the urine after it has been rapidly absorbed.

To test the possibility of adaptation to high intake levels, molybdenum-99 was also given to rats which had been on molybdenum in their drinking water at 0, 10, 100, and 1,000 mgMo/L since birth (126,133). Those on the higher levels (100 and 1,000 mgMo/L) eliminated the molybdenum-99 faster than those on 0 and 10 mgMo/L. This is of interest in view of results shown in Figure 8

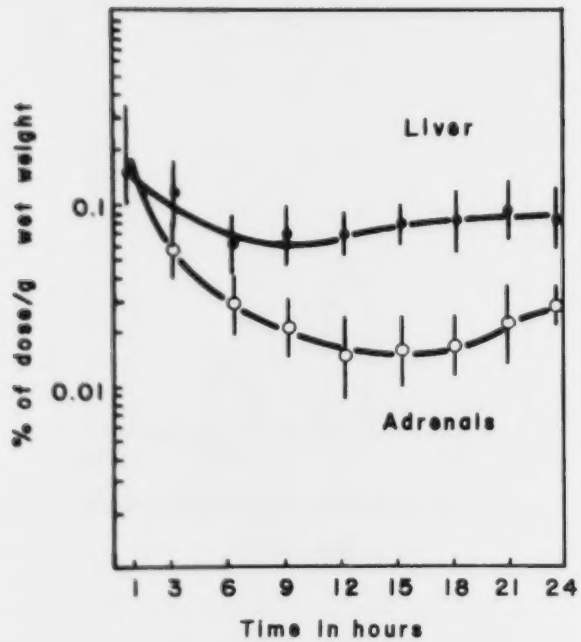

Figure 7. Percent of molybdenum-99 dose accumulated per gram of tissue (wet weight) for liver and adrenals versus time after dosing.

where accumulation is higher at 10 mgMo/L than at 100 mgMo/L indicating that there is an adaptation to the high intake.

Little is known of the transport of molybdenum in the blood and its storage in tissues. Bibr and Lener (134) tested two of the oxidation states of molybdenum expected to be found in biological systems. (Molybdenum-V exists in the molybdate-cysteine complex while Mo-VI is the oxidation state found in the molybdate ion.) They showed that, at most levels, Mo-V binds to many of the plasma globulins while Mo-VI binds to almost none. This indicates that there may be a difference in the way that molybdenum from food (possibly Mo-V) and from water (Mo-VI) are carried in the blood. If this is true, then uptake, residence time, and elimination of molybdenum by cells may be different for the two forms. Furthermore, all of the absorption studies cited above were performed with molybdate, the inorganic form, but nothing is known about the bioavailability and absorption of organically bound molybdenum. It is possible that much of the organically bound molybdenum exists as the molybdate-cysteine complex, the form in which molybdenum is found in all the known molybdenum bearing enzymes (135).

Inorganic and organically bound molybdenum may differ in their bioavailability and metabolic behavior. Since these differences may lead to different

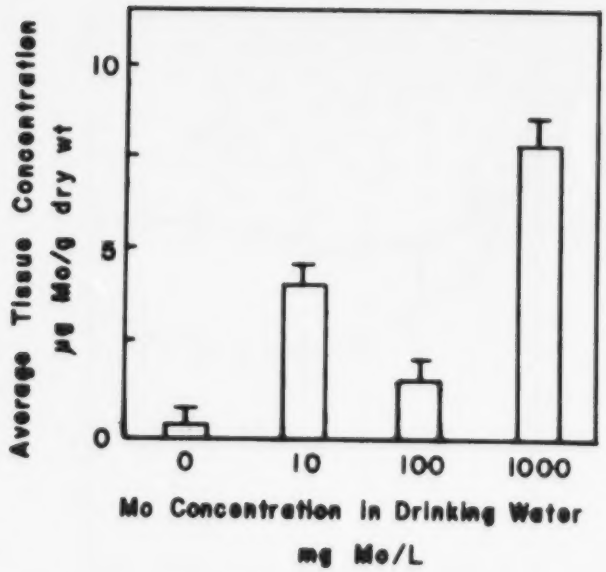

Figure 8. Weighted average molybdenum concentration of ten tissues (dry weight basis) from male rats receiving indicated molybdenum concentrations in their drinking water.

conclusions regarding potential detrimental effects of excess molybdenum, additional studies of various chemical forms of molybdenum should be performed.

The only facts known about the storage of molybdenum is that apparently large amounts of active and inactive xanthine and sulfite oxidases are stored in the liver (131). Johnson and Rajagopolan (131) depleted rats of molybdenum by feeding them tungstate over several weeks. During this time liver xanthine oxidase and sulfite oxidase activities decreased more rapidly than did the total molybdenum in the liver. This discovery led to the suggestion that there is an unknown non-protein form in which molybdenum is stored.

In summary, the inorganic molybdate which exists in drinking water is rapidly and nearly completely absorbed. It moves into and out of most tissues very rapidly, and it is excreted in the urine at nearly the same high rate as it is absorbed. Animals receiving elevated levels of molybdate rapidly attain balance and there is no progressive accumulation of this substance. Much still remains to be learned of the physiology of molybdenum in humans and other non-ruminants.

TISSUE DISTRIBUTION IN ANIMALS

Data were gathered on the levels of molybdenum in 10 selected tissues of rats given the element chronically at 0, 10, and 100 mgMo/L in their drinking water. X-ray fluorescence analysis was used to determine the molybdenum

concentration of tissues obtained from adult rats. Figure 8 shows the average tissue concentration plotted against intake levels. It can be seen that animals on 0 and 100 mgMo/L in their water exhibit lower tissue concentrations than animals on 10 and 1,000 mgMo/L. The expected dose-response effect was therefore not observed. Adaptation to the higher levels has been suggested as an explanation for this phenomenon (133). It should be noted that the average values given here do not represent whole-body burdens but are biased toward high concentrations because we sampled all tissues which were previously found to be high in molybdenum. Only two low-concentration tissues, muscle and brain, were included. Whole body analyses performed in this laboratory show smaller differences between the dose groups. However, the same basic pattern is found. The molybdenum concentrations found in the tissues of the rats receiving drinking water containing 10 mgMo/L are similar to those found by Fairhall and others (111) in guinea pigs which had been given 50 mg of Mo as MoO_3 by oral administration with a syringe.

The tissue concentrations in the controls were very similar to those seen by other workers. In general, rats on a normal dietary intake of molybdenum have a higher molybdenum concentration in their livers than in most other tissues (kidney, spleen, brain, muscle, etc.) (132,136). Similar results have been found for human tissues. At greatly increased molybdenum intakes the highest molybdenum concentrations are found in the kidneys of the exposed animals rather than the livers. This result has also been obtained for guinea pigs (117) and goats (50). In addition, Table 13 also shows that there is considerable uptake by the testes. This result is potentially significant in

TABLE 13. MOLYBDENUM CONCENTRATIONS (PPM DRY WEIGHT BASIS) IN TISSUES OF RATS GIVEN DIFFERENT LEVELS OF MOLYBDENUM AS Na_2MoO_4 IN THEIR DRINKING WATER

Mo in water mgMo/L	0	10	100	1,000
Adrenals	0.57±0.09 (13)	19±2.9 (14)	4.3±0.41 (10)	40±5.7 (10)
Brain	0.2±0.06 (13)	2.4±0.31 (15)	0.79±0.1 (10)	5.4±0.6 (10)
Muscle	0.12±0.6 (20)	2.4±0.8 (16)	1.1±0.3 (12)	7.2±0.35 (10)
Kidney	1.7±0.3 (21)	54±9.6 (15)	18±4.3 (12)	77±9.2 (10)
Liver	2.2±0.12 (13)	15±3.9 (15)	4.8±0.52 (10)	22±4.1 (10)
Testes	0.5±0.6 (19)	38±5.2 (13)	6.9±1.4 (10)	76±8.5 (10)

view of the fact that increased sterility was observed in the male rats receiving 1,000 mgMo/L in their drinking water. Molybdenum-induced sterility has been previously reported in male rats (137,138) and cattle (139). Similar increases in molybdenum concentration in the ovaries of goats have been reported. The only research which has indicated a possible effect on the reproductive function in female animals was reported by Schroeder who found significantly increased rates of young deaths and dead litters in rats on 10 mgMo/L in their drinking water (140). These results will be discussed further in Section 8, Toxicity-Animals.

SECTION 8

BIOLOGICAL EFFECTS

TOXICITY - ANIMALS

Introduction

While conclusive evidence that molybdenum is essential to animals has not been reported, there is widespread agreement among researchers that this element is indeed essential (67). For essential elements there are three ranges of biological effects--deficiency, adequacy, and toxicity. While these ranges have not been well-defined for molybdenum or any other essential trace elements, Mertz (141) has proposed a tentative dose-response curve which is shown in Figure 9. Because of the type of behavior exhibited in this curve, it is very difficult to study the toxicological properties of an essential trace element such as molybdenum, zinc, copper, cobalt, and nickel. Moreover, environmental exposures to these elements are rarely at the extreme ends of the curves, although acute toxicity may occur from occupational exposures. Because food sources contain variable amounts of essential trace elements, and because the concentrations in plants are determined by various geochemical parameters such as pH, it is reasonable to assume that animals can adapt to moderate changes in daily intake.

Thus, the problem of defining a safe dietary intake for essential trace elements such as molybdenum is very difficult. It is clear that the "no intake" option is not acceptable. On the positive side, it is also clear that there is a "level" below which no toxic effects will occur whereas conclusive evidence for such a "no effect" level is lacking for non-essential toxic substances. Of course, as intake is further reduced deficiency symptoms may occur. The problem is to define this "no effect" level.

Friberg and others (50) recently reviewed the research on the toxicity of molybdenum. One characteristic of the toxicological properties of molybdenum is the great variability from species to species both in terms of the toxic doses and the effect. Cattle are by far the most susceptible (67), while sheep are somewhat less tolerant; horses and pigs are the most tolerant of farm animals. Rats, guinea pigs, and rabbits are intermediate between sheep and horses.

Livestock

The most common clinical disorder in livestock due to high molybdenum intake is known as molybdenosis (or teart, or peat scours in some areas). This disease is characterized by diarrhea (scouring), discoloration of hair, loss

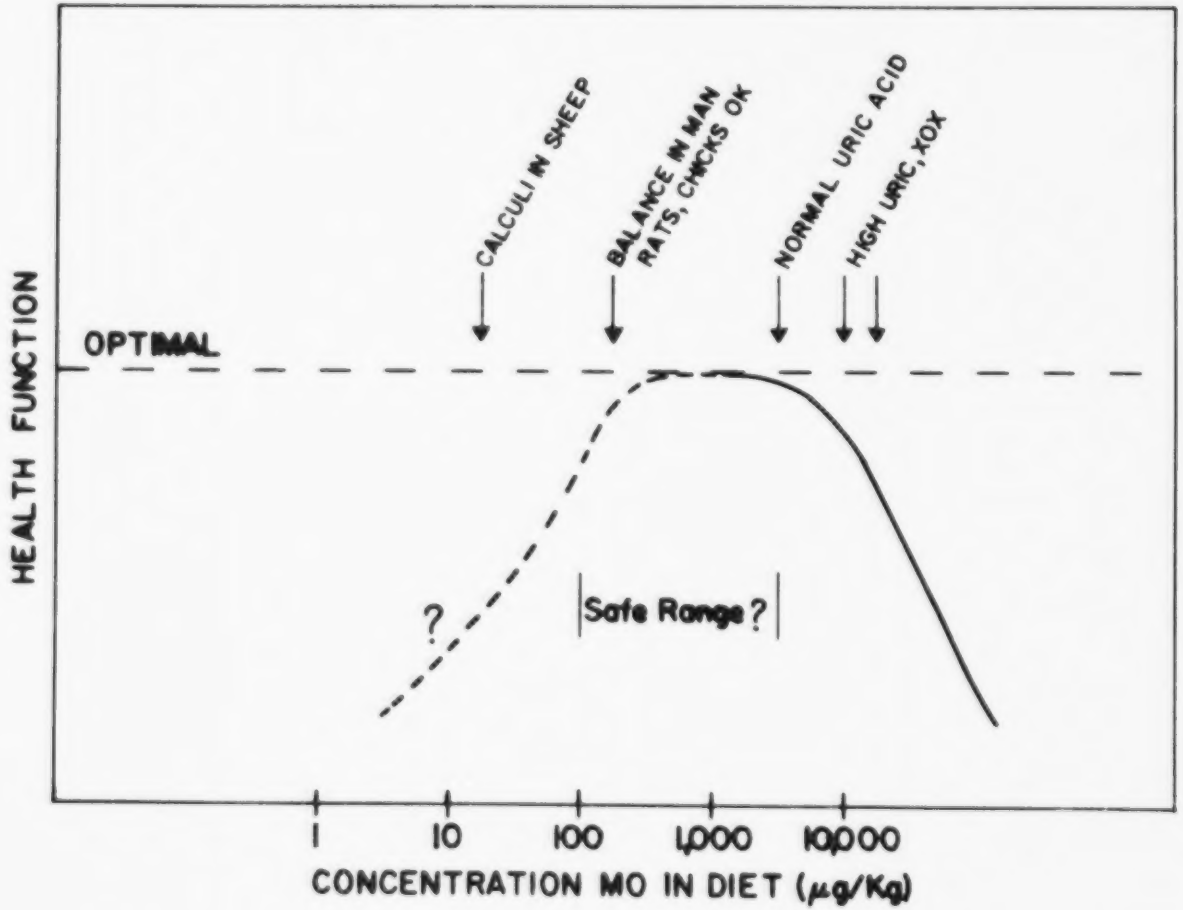

Figure 9. Hypothetical dose-response curve for molybdenum [after Mertz (141)].

of appetite (aneroxia), joint abnormalities, osteoporosis, reproductive difficulties, lack of libido, testicular degeneration, and occasionally death (67). Forage molybdenum concentrations of 20 to 100 ppm Mo can induce these symptoms (67). The minimum level depends upon the copper status of the animal's diet as well as several other factors. Normal plants contain 1 to 2 ppm Mo.

It has been shown in cattle and sheep, and some other species, that the excess molybdenum leads to a copper deficiency that can often be reversed by dietary copper supplementation (67). Moreover, the amount of molybdenum required to cause a toxic effect is strongly dependent on the amount of copper in the diet. Sulfate has also been shown to interact with molybdenum and copper (67).

Miltimore and Mason (142) have suggested that a copper/molybdenum ratio of 2:1 represents a critical level and that molybdenosis can be anticipated if the ratio falls below 2. Alloway (143), on the other hand, believes the critical ratio may be closer to 4:1.

At lower levels of molybdenum intake subclinical effects in cattle have been reported by Thornton and others (144). They have described the occurrence of decreased weight gains, decreased fertility, and delayed maturity in cattle grazing on forage containing 5 ppm Mo and 10 ppm Cu. These cattle showed no clinical manifestations of molybdenosis.

Another clinical disorder in livestock (sheep) which has been reported to be associated with molydenum is "swayback," which is neonatal enzootic ataxia encountered in lambs (145). This disorder involves the demyeliniation of the cerebrum in congenital cases and in the spinal cord for postnatally acquired cases. The mechanism involved in this pathology appears to be an insufficient production of cytochrome oxidase (146,147). A similar myelin anomaly has been reported in copper-deficient rats (148).

All ruminants are not as sensitive as cattle and sheep. Concentrations as high as 1,000 ppm to 7,000 ppm Mo in the diet were required to produce toxic effects in deer (growth reduction)(149). These results demonstrate the wide variation in toxicity of molybdenum among different species.

Laboratory Animals - Acute Toxicity

Considerable research has been done on the toxicity of molybdenum to guinea pigs, rats, mice, and rabbits. These animals are much less sensitive than cattle and sheep. As with cattle and sheep, the level at which molybdenum toxicity is observed depends on the copper status of the animal.

The symptoms vary from species to species. In young rabbits the effects of molybdenum toxicity are loss of appetite, loss of weight, baldness, and dermatosis (150). In rats and guinea pigs, loss of appetite, retardation of growth, loss of body weight, and sterility have been reported (138,151).

For cattle a lethal dose of molybdenum is on the order of 10 mgMo/kg body weight (152), whereas for rats (153) it is about 100 to 150 mgMo/kg body weight and for guinea pigs (117) 250 mgMo/kg body weight. Thyroid function was

drastically reduced in rabbits at 5,000 ppm Mo in the food (154). This concentration was lethal to rats (155). Mortality was also produced in our laboratory by force-feeding rats a daily equivalent of 2,500 ppm Mo (about 50 mgMo/day at the ingestion rate of 20 mL H_2O or 20 g food). Mortality was 100% in 10 days. Acromotrichous anemia was the most obvious effect. Liver and kidneys, normally dark red, were almost white in all cases.

Rats exhibit relatively mild effects at lower intakes (118,133) as long as they are well-cared for. Rats given molybdenum as molybdate at 250 or 1,000 mgMo/L over their lifetime in their drinking water did not exhibit any severe toxicity symptoms. Rat pups from dams that had been on 1,000 mgMo/L most of their lives were maintained continually on the same intake. This exposure produced stunting, rough hair, sterile males, and some hyperactivity, but it did not shorten the life span noticeably. Females produced pups which were reduced in size (40% to 50% of normal); however, the number of pups was only slightly less than from untreated dams (Table 14). The maternal performance of these animals was similar to rats on much lower levels. The number of abnormal births, still-born, and resorbed fetuses was within normal limits, and there were no indications of increased incidence of tumors or teratogenic effects. Other workers (118) have found similar results for rats on 500 and 800 ppm Mo in their food. At intermediate dose levels (50, 100, 250 ppm Mo) growth rates were significantly reduced but not as dramatically as at 1,000 mgMo/L (Table 15) and litters were somewhat smaller than in controls. Otherwise there were no obvious clinical symptoms of toxicity.

TABLE 14. EFFECT OF MOLYBDENUM ON LITTER SIZE IN WHITE RATS

Mo in water (mg/L)	0	10	100	1,000
N	17	25	16	6
Pups/litter	11.2±0.5	12.4±0.3	8.6±0.9	10.0±0.56
Range	8 - 15	6 - 16	2 - 14	9 - 12

The most consistent effect of high molybdenum intake in rats is the depression of growth or weight loss. Since this is usually accompanied by anorexia, it has been suggested that the weight loss is due to reduced food intake rather than some other metabolic process. Some others suggest that the rats develop an ability to recognize the presence of molybdenum in the diet and voluntarily reject the food (156). This hypothesis has been disputed by Arrington and others who reported pair-feeding experiments which demonstrated that reduced food intake was not the primary cause of stunting (155).

It was mentioned previously that male rats whose mothers were on 100 to 1,000 mgMo/L in their water and who themselves were on the same level showed a high incidence of sterility. The effect of excess molybdenum on male fertility has been reported earlier in both calves and rats. In a study reported by Jeter and Davis (138) Long-Evans rats received varying amounts of sodium molybdate in their diets. Seventy-five percent of the males which were fed

TABLE 15. REPRESENTATIVE WEIGHTS FOR RATS ON VARIOUS DIETARY REGIMES

Defined diet Mo ppm	Fully Exposed 61 days	From weaning 56 days
0.5	326±12.9 (8)	245±14* (8)
10	267±15.1 (8)	261±13* (8)
Mo in water mgMo/L		**60 days**
0	251±9.6 (15)	250±15.4 (8)
10	202±12* (30)	245±10.4 (8)
100	210±14.1* (21)	242±14.5 (8)
1,000	108±7.9* (15)	
Mo in water mgMo/L		**76 days**
0		329±6.5* (8)
5		316±5.9* (8)
10		312±5.4* (8)

*Different from 0 mgMo/L group at p = <.05. Values without * are not statistically different from controls.

from weaning on a diet containing 80 ppm Mo and 140 ppm Mo were found to be sterile. The limited histological examinations which were performed showed varying degrees of seminiferous tubule degeneration in the rats receiving high doses of molybdenum. Thomas and Moss (139) found severe degeneration of the seminiferous tubules in male calves which were given a diet containing 300 ppm Mo to 400 ppm Mo (4 to 8 mgMo/kg body weight) over 130 days. The calves were also reported to show a marked decrease in libido. In a part of Section 7 (Tissue Distribution in Animals) data were presented showing that molybdenum is readily accumulated in the testes of rats.

Some of the effects of high molybdenum intakes (equivalent to about 100 ppm Mo in the diet or more) that have been reported are summarized below:

Rats	Rabbits
Loss of appetite	Loss of appetite
Loss of weight	Loss of weight
Rough coat	Alopecia
Growth retardation	Dermatosis
Anemia	Anemia
Mendibular exostoses	Skeletal deformities

Rats	Rabbits
Bone deformities	Joint deformities
Histopathology of liver and kidney disease	Decreased thyroxin
Increased liver copper	
Increased xanthine oxidase	
Increased uric acid	
Impaired alkaline phosphatase and sulfide oxidase activity	
Male sterility	

Laboratory Animals - Chronic Toxicity

While considerable research has been done on the effects of very high levels (>100 ppm) of molybdenum in the diet, considerably less work has been performed on long-term, low level exposure. As noted in Section 5, the concentration of molybdenum in water rarely exceeds 1 µgMo/L and the highest level observed outside of an industrial water was 50 mgMo/L. Thus, it is important to study the effects of levels of molybdenum corresponding to perhaps 5 to 10 ppm Mo in the diet.

Schroeder (140,157) has studied reproduction effects in mice receiving 10 mgMo/L in their water and 0.25 ppm Mo in their food. He found significantly increased rates of young deaths and dead litters in this group after three generations. The controls received the same food with 1 mgMo/L in the water. His findings suggested the following order of toxicity with respect to reproduction:

$$Hg > Cd > Pb > Se > Mo > Ti > Ni > As.$$

Suttle (158) fed animals a copper deficient diet, thereby depleting their copper stores. He then supplemented the diet with copper and various levels of molybdenum and measured the rate of copper repletion. In guinea pigs he found a significant reduction in response in the group fed 4.5 ppm Mo in their diet as opposed to the controls which received 0.5 ppm Mo in their diet. Moreover, he found that the influence of molybdenum on copper utilization was not linear. In fact, the first increment of 4 ppm Mo gave as great a reduction in copper utilization as the change from 25 to 100 ppm. Thus, small increments seem to have disproportionately large effects. Somewhat similar results have been found in our own work which is reported below. Suttle suggested that the copper-molybdenum interaction may have a greater significance for non-ruminants, including man, than has previously been suggested.

In our own work molybdenum at 5 and 10 ppm produced deleterious effects on some animal functions. No additional animal treatments were required to produce these effects. However, effects on other animal functions were only observed when the animals were subjected to stress. The application of stress to experimental animals was chosen to amplify the effects of subclinical toxic doses. The working hypothesis is that the potential toxicant will alter the animals' ability to adapt to an externally applied stress, or that stress will intensify the otherwise subclinical effect of the toxic substance and clinical

symptoms will be expressed at normally subclinical doses. In addition, stress produces a measurable response in several physiological activities. Objective physical measurements simplify the quantification of the response to experimental variables. Examination of the stress response is also useful in relating the laboratory animal experiments to humans since humans are subjected to a wide range of stresses.

An experimental protocol was developed to test the rats. Several different stresses were applied and their effects on various physiological and behavioral activities were measured (122). Male, Sprague-Dawley rats were either (a) raised from dams maintained on identical test diets (fully exposed) or (b) bought as weanlings (3 weeks old) and started immediately on the test intake of molybdenum. Physiological and behavioral tests were begun at 7 weeks and continued until sacrifice at 17 weeks. Oxygen consumption was measured on sleeping rats once each day for four days. After this, behavior and activity were tested in an Open Field arena (159) once a day for four more days. Each animal was then subjected to a non-destructive psychological stress (fear) and the immediate response was measured. The entire sequence was repeated separately for oxygen consumption measurements and for Open Field testing. The determination of oxygen consumption proved to be a useful measure of general body metabolism and health. The behavioral tests were expected to be sensitive indicators of impaired neural function.

At 15 weeks the rats were subjected to 4 days at $4^{o}C$ to $5^{o}C$, and at 17 weeks they were kept at $37^{o}C$ for 16 hours and then sacrificed. Tests carried out and data gathered will be discussed below. By using several different approaches it was possible to identify some of the subtle effects of excess molybdenum.

Growth--

The effects of possible toxicants on growth is a common measure of toxicity (160). A significant reduction in growth was observed for rats receiving 5 mgMo/L to 10 mgMo/L (in the form of sodium molybdate) in their drinking water (Table 15). However, these concentrations of molybdate in the animals' food produced no effect. This difference can be explained by the difference in total molybdenum ingested. (A rat receiving 10 ppm Mo in food ingests about 250 µgMo/day while a rat receiving 10 mgMo/L in water ingested 350 to 400 µgMo/day from water and 25 µgMo/day from its food.) Weight losses diminished as the rats' weights approached 500 to 600 grams. For these larger rats doses of 1,000 mgMo/L were required for significant weight differences.

Tests on Unstressed Rats--

Oxygen Consumption ($\dot{V}O_2$)--The sleeping and awake unstressed metabolic rates (measured by oxygen consumption) of animals receiving 10 mgMo/L in water were significantly higher than control animals (no added molybdenum--see Fig. 10). Oxygen consumption was also elevated in animals receiving 50 mgMo/L and 100 mgMo/L. However, no clear dose-response effect was observed since the oxygen consumptions were only slightly higher than that for the 10 mgMo/L animals. This result is similar to Suttle's results in this phenomenon. However, we believe that the toxicity of molybdenum is not simply a function of the copper to molybdenum ratio as proposed by Miltimore and Mason

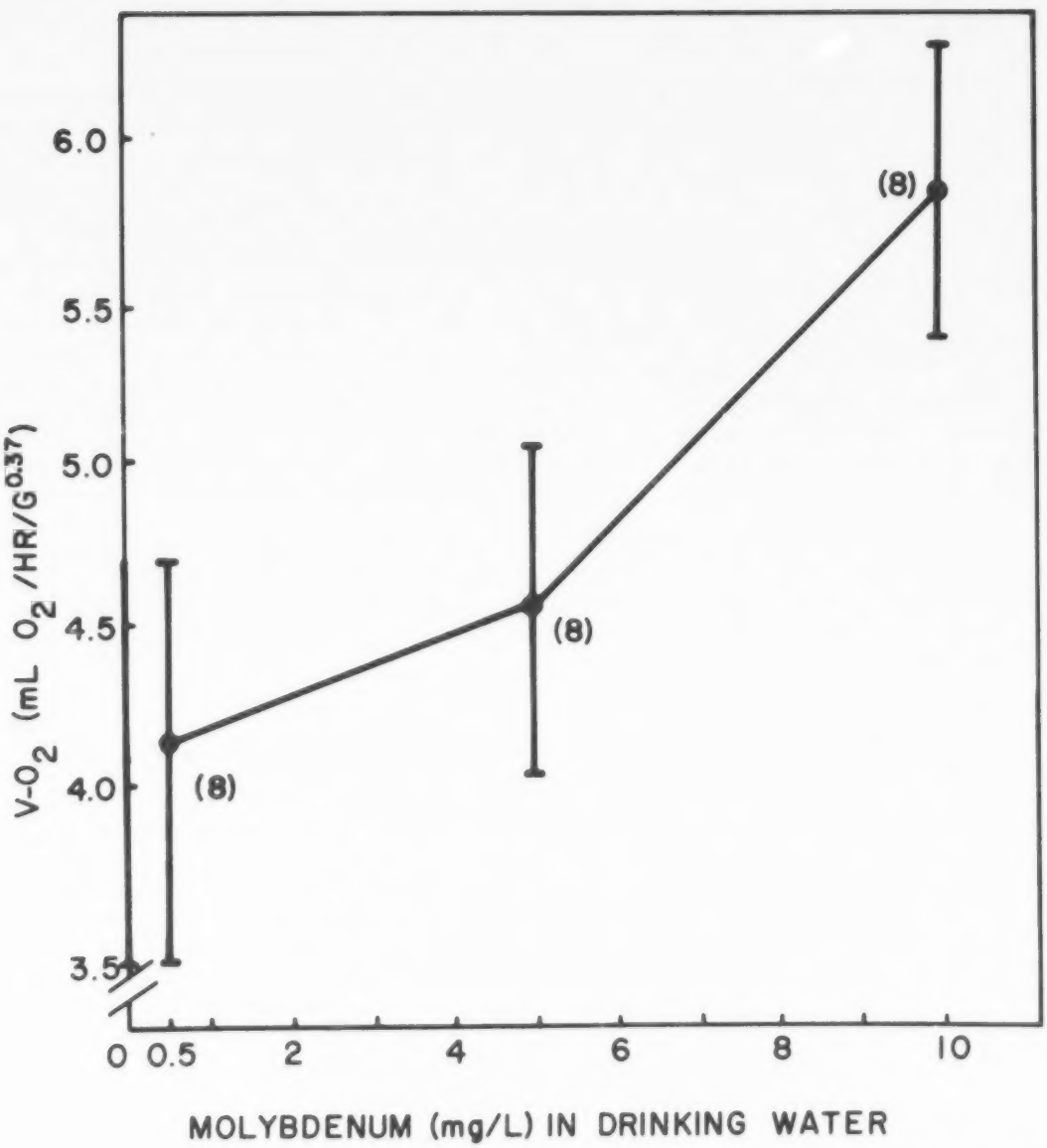

Figure 10. Oxygen consumption in sleeping rats versus molybdenum concentration in drinking water. The numbers in parentheses are the number of rats in each test group.

(142), but that it also depends on the dietary copper intake in the sense that there is a threshold or "adequate" value. When copper intake falls below that value the animal is much more susceptible to molybdenum toxicity than when the copper intake is more than adequate. Since the rats which received the higher levels of molybdenum (50 to 100 mgMo/L) all received adequate copper (7 to 10 ppm Cu), the effect of the additional molybdenum exposure was reduced.

The largest elevation above the controls was obtained with a group of rats on food low in copper and elevated in molybdenum (2.5 and 10 ppm, respectively). In these animals, the sleeping $V\text{-}O_2$ was nearly double that of the control group receiving 10 ppm Cu and 0.5 ppm Mo (161). In an attempt to explain this result we examined the fine structure of the adrenal cortex of animals receiving 0, 10, and 100 mgMo/L in their drinking water. The animals receiving the highest level showed statistically higher numbers of mitochondria in their cells when compared to controls. Animals receiving 10 mgMo/L also had higher mitochondria counts but the numbers were not statistically significant (162). Thus, the larger number of mitochondria could result in higher oxygen consumption.

Open Field--Activity levels (number of squares entered) in the Open Field were higher for unstressed rats receiving 10 mgMo/L than control animals (see Fig. 11). No difference was observed for animals receiving 5 mgMo/L. Rats on 10 mgMo/L increased their activity considerably during the last three days of the five day trials. This was not true for the control rats (163). In Open Field testing it is commonly observed that rats will settle down to consistent activity in trials two through five, but molybdenum appears to have modified this result. This behavior may be the result of the generally higher metabolic rate.

Plasma Glucose--Glucose is mobilized under stress by epinephrine and corticosteroids. Copper-containing enzymes are important in the synthetic pathways of both of these hormones (164). Therefore plasma glucose was measured as a possible indicator of molybdenum toxicity. The plasma glucose levels for unstressed experimental animals and controls were similar (average 155 mg%). However, stressed animals receiving elevated molybdenum showed significantly lower plasma glucose levels compared to stressed controls on normal levels of molybdenum. The largest reductions were observed with animals receiving the lowest copper levels (2.5 ppm).

Ceruloplasmin--Ceruloplasmin is a copper-containing enzyme which is synthesized in the liver. The enzyme is found in plasma, and under stress it can be mobilized from liver stores. Because of the known interactions between molybdenum and copper (165), the activity of this enzyme was measured as a potential indicator of toxicity.

Molybdenum at 5, 10, and 50 mgMo/L in water had no effect on the ceruloplasmin activity in rats eating Lab Chow. This food contains 7 to 10 ppm Cu and this concentration appears to be adequate (164). Animals receiving a defined diet low in copper (2.5 ppm) and excess molybdenum showed significantly lower ceruloplasmin activity ($p<0.001$) (Fig. 12). Animals receiving 10 ppm Cu and excess molybdenum also showed slight reductions in ceruloplasmin

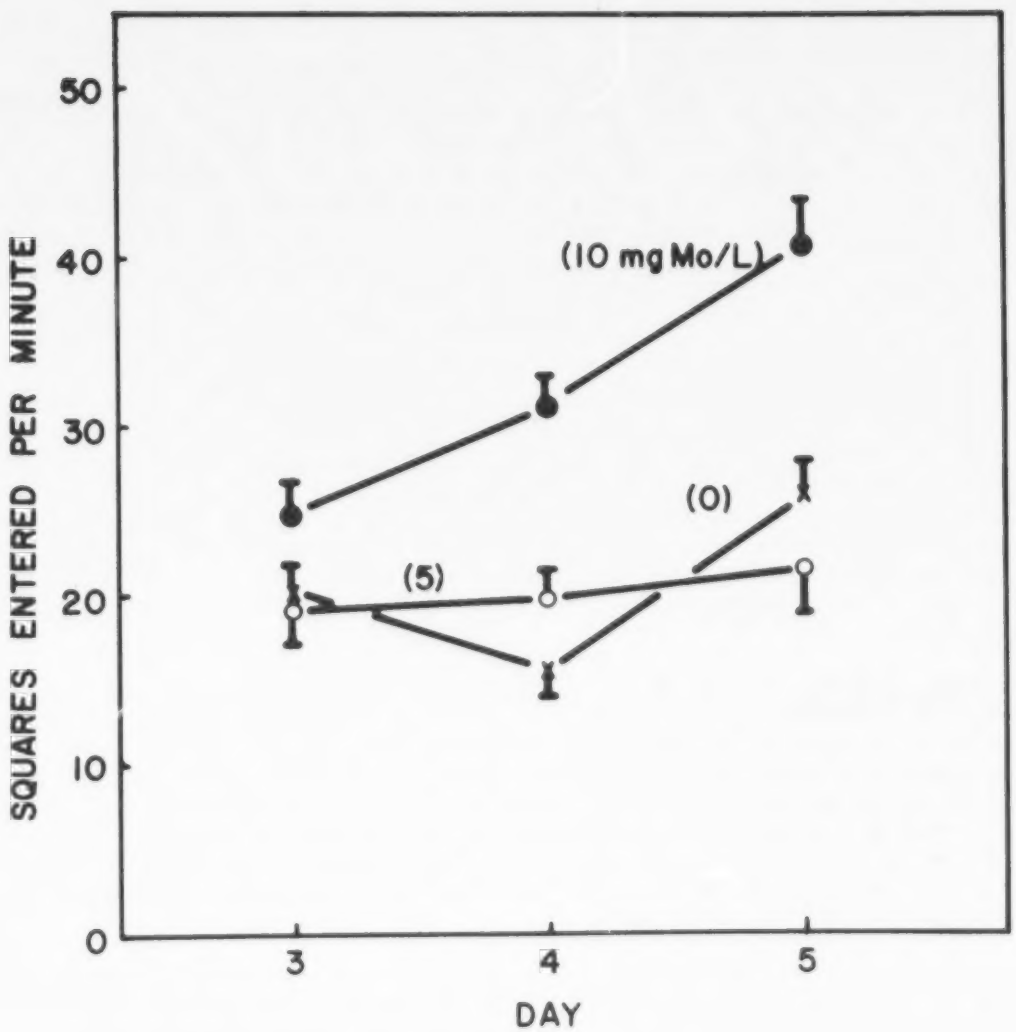

Figure 11. Unstressed animal activity as measured by the number of squares entered per minute in an open field arena. The rats received 0, 5, or 10 mgMo/L in drinking water. Eight animals in each group were tested on five successive days. By convention, the results of the first two days were not recorded.

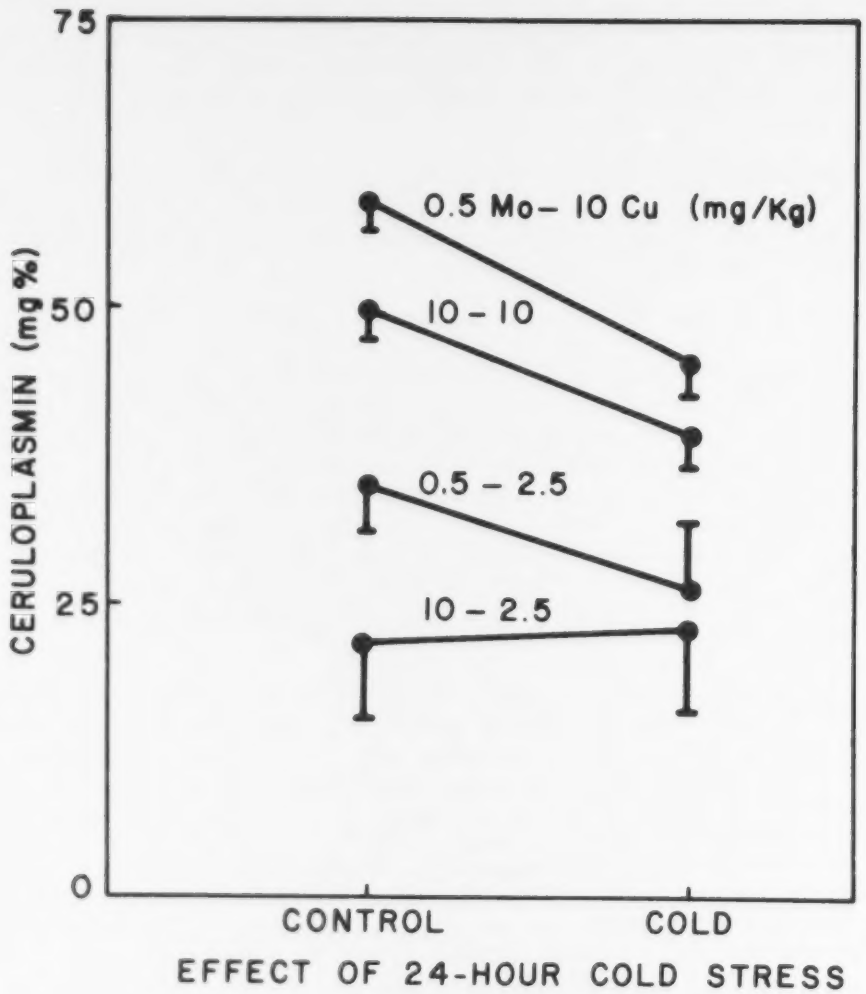

Figure 12. Effect of cold stress on serum ceruloplasmin in rats given molybdenum and copper in a defined diet at the indicated levels. Vertical bars show one standard error. There were eight animals in each test group.

activities ($p=0.05$). These results suggest that copper must be below "adequate" in order for increased molybdenum to measurably reduce ceruloplasmin activity.

Hematology--The appearance of achromotrichous anemia in rats receiving elevated molybdenum levels suggested that some hematological factors might be affected. However, 5, 10, 50, 100, or 1,000 ppm Mo in food or water produced no significant effects on hematocrit, hemoglobin, or blood cell numbers. While one study (165) did report anemia in rats receiving 400 ppm Mo in their diet (as sodium molybdate) other studies (118) have failed to reproduce this result.

On the other hand, there is a rather consistent picture of hematological complications in rabbits on high doses of molybdenum. Several authors have reported decreased hemoglobin and hematocrit in rabbits on rations containing 1,000 to 4,000 ppm Mo (150,165,166).

Tests on Stressed Rats--

Drop Stress--The drop stress is a short-term non-destructive fear stress which was expected to amplify the effects of molybdenum on sympathetic nervous system activity via altered secretion of catecholamines. After repeated free-fall in a cage, the metabolic response consists of a rapid decline in oxygen consumption followed by a rapid rise to more than double the rate observed for the sleeping animal. After the peak consumption rate is attained the rate decreases for 15 to 20 minutes until the animal reaches its awake resting rate. Rats (bred from dams on the same level) exposed to 10 mgMo/L from conception showed shorter total response times than controls ($p<0.05$). No other measured parameters showed significant effects (161).

Open Field Behavior--Rats were subjected to a similar drop stress and immediately placed in an open field area for a four minute test. The open field test was repeated (the stress was not repeated) 24 hours later and four days later to determine any persistent effects of the original stress. The immediate response to the drop stress for all animal groups was a large reduction in activity (squares entered). The activity in all groups decreased to about the same level and no significant effects of molybdenum were found on any aspects of behavior. There was no indication of memory impairment when the tests were repeated. Other tests of memory, using a different protocol, also showed that excess molybdenum had no effect.

Summary of Results--

Some deleterious effects of molybdenum were found at 5 and 10 ppm Mo. The animals showed reduced growth, increased metabolism as measured by oxygen consumption, and increased activitv as measured by open field tests. Some of the deleterious effects were brought out by application of stress. In all cases the excess molybdenum produced a reduction in the animals' stress response. No simple relationship between dose and response was observed. Evidence exists for an adaptation at higher molybdenum doses (near 100 ppm).

Some of the deleterious effects appeared only when the animal was receiving a diet which was low in copper. Results obtained in these experiments

indicate that the copper:molybdenum ratio is not sufficient for predicting the response to excess molybdenum. There exists a minimum adequate copper concentration below which relatively small increases in molybdenum intake can induce adverse effects. Excess molybdenum produced no effect on memory or hematological measures such as blood cell counts, hematocrit, or hemoglobin.

The results of the tests performed on the rats are shown in Table 16. The designations low and high dietary molybdenum correspond to 1 ppm Mo or less and 5 ppm Mo and 10 ppm Mo, respectively. A plus sign (+) indicates an increase in the measured parameter and a minus sign (-) indicates a decrease. A zero (0) indicates no significant difference from control. The effect of molybdenum on ceruloplasmin activity and plasma glucose concentrations showed a dependence on dietary copper intake. These variables are indicated where relevant. (Low copper is 2.5 ppm; higher copper is 7 to 10 ppm.)

TABLE 16. SUMMARY OF TEST RESULTS

Open Field Test
(Activity)

	Unstressed	Drop stressed
High Mo	+	-
Low Mo	Control	-

Metabolism
(Oxygen consumption: $V\text{-}O_2$)

	Unstressed	Drop stressed
High Mo	+	++
Low Mo	Control	++

Plasma glucose
(Concentration in mg%)

	Unstressed			Heat stressed
			Short term	Long term	
High Mo	0	High Cu	-	NA	0
		Low Cu	+	0	
Low Mo	Control		+	+	+

(continued)

TABLE 16. (continued)

	Ceruloplasmin (Activity of enzyme)			
	Unstressed		Drop stressed	
	High Cu	Low Cu	High Cu	Low Cu
High Mo	-	--	-	--
Low Mo	Control	--	-	--

Summary

The toxicological properties of molybdenum are very complex. No doubt this complexity is caused in part by the role of molybdenum as an essential trace element, thus leading to a more complicated picture than is the case for a substance which does not play such a role.

The most susceptible species are generally ruminants and in particular, cattle and sheep. Since the mid-1930's when molybdenum was shown to be the cause of a long-recognized disease in cattle known as "peat scours" or "teart," many cases of molybdenosis in livestock have been reported. In most cases the sources of the excess molybdenum were natural. But in some cases, industrial activity was the cause. In most cases the symptoms--which are normally diarrhea, emaciation, and male sterility--can often be reversed by the addition of copper to the animal's diet.

The interaction among molybdenum, copper, and sulfate has been studied in several species. One hypothesis has been that a Cu-Mo-S compound is formed which renders copper unavailable to the animal (167,168).

Rats, rabbits, and guinea pigs are much less susceptible to molybdenum toxicity than cattle and sheep. Whereas clinical effects can be induced in cattle grazing forage containing 10 to 20 ppm Mo (normal plants contain 1 to 2 ppm Mo), 100 to 400 ppm Mo in the diet is required to induce clinical manifestations in rats. The symptoms vary from species to species and include: weight loss, growth reduction, skeletal deformities, and sterility in males.

Subclinical effects have been reported in rats, mice, and guinea pigs receiving dietary levels that are 5 to 10 times normal (the equivalent of 5 to 10 ppm Mo in food). These consist of reproductive effects, effects on copper metabolism, growth retardation, and a generally lowered response to stress.

These results indicate that for several species the upper end of the range of sufficiency or the safe range is about 5 to 10 times the "normal" level. Since no diets have yet been devised which are so low in molybdenum as to induce a deficiency, the lower end of the safe range (that is, the minimum daily requirement) is as yet unknown.

HUMAN DIETARY INTAKES AND NUTRITIONAL REQUIREMENTS

It is estimated on the basis of balance studies (169-171) that 30% to 80% of the molybdenum consumed is absorbed. Based upon urinary excretion of subjects in our studies, we estimate the minimum absorption of molybdenum from food and water to be between 25% and 30% of the intake. It is not known whether molybdenum in drinking water is absorbed more rapidly than that contained in food. Little information is available on the relative amounts of molybdenum absorbed by humans under varying conditions or the influence upon molybdenum absorption of other components of the diet, particularly protein and other trace elements. The discussion on Tissue Distribution in Animals in Section 7 presents information on the absorption of molybdenum which has been derived from rat and other animal studies.

Water is usually considered to be a minor factor in the intake of molybdenum and many other trace elements (60,72,82). If we assume a molybdenum intake from food at about 180 µgMo/day (from our work), persons consuming one to two liters of water per day which contains less than 10 µgMo/L will not derive a significant portion of their daily molybdenum intake from their drinking water. If a person consumes water containing 40 to 50 µgMo/L, such as in the Denver metropolitan area, then the water contributes one-fourth to one-half of the daily molybdenum intake. If we assume a food intake of 180 µgMo/L, and a water supply containing 180 µgMo/L, as in some mountain areas of Colorado, then the water may contribute one-half to two-thirds of the daily molybdenum intake. Further information on the amounts of molybdenum present in drinking water is presented in Section 6, Water.

Based upon the work of a number of investigators, Friberg and others (50) state that the usual intake of molybdenum via the diet can vary between 100 and 500 µgMo/day.

Investigators in the U.S.S.R. have estimated the daily intake of molybdenum from the diet at 329 to 376 µgMo/day for adults (172), 156 to 161 µgMo/day for children (173), and 200 to 500 µgMo/day for children and adults (174).

On the basis of total diet samples from different areas of the United Kingdom, Hamilton and Minski (175) reported a mean daily intake of 128 µgMo/day.

Schroeder and others (132) estimated that the average diet in the United States contained 335 µgMo/day, with a range between 210 and 460 µgMo/day, based on analyses of samples of complete hospital diets.

Wester (176) examined duplicate samples of the hospital diets of two patients, during six periods of five days each. Average daily intakes for each period varied between 250 and 1,000 µgMo/day.

Tipton and Stewart (177) found daily intakes averaging 110, 120, and 460 µg Mo for three subjects in a balance study. Wester (170) studied four healthy subjects in a 10 day metabolic study. The daily intake of molybdenum varied between 115 and 245 µg Mo. Robinson and others (171) found a daily

intake in New Zealand of 46 to 96 µg Mo in a metabolic study with a diet based on meatloaf.

Our study (76) has found the average daily intake of molybdenum in the United States to vary between 120 and 240 µgMo/day, depending upon age, sex, and family income. We estimated the average daily intake of molybdenum via the diet in the United States to be 180 µgMo/day, based on a market basket sampling program, and a method of estimation using U.S.D.A. published estimates of food consumption (77). Figures 13 and 14 show our calculated daily molybdenum intake for each age group and the daily molybdenum intake for each age group per kg body mass. The intake by males was generally greater than that by females. Persons with a lower annual family income generally had a higher intake of molybdenum per day, which was assumed to be due to the greater proportion of legumes and cereals making up their diets.

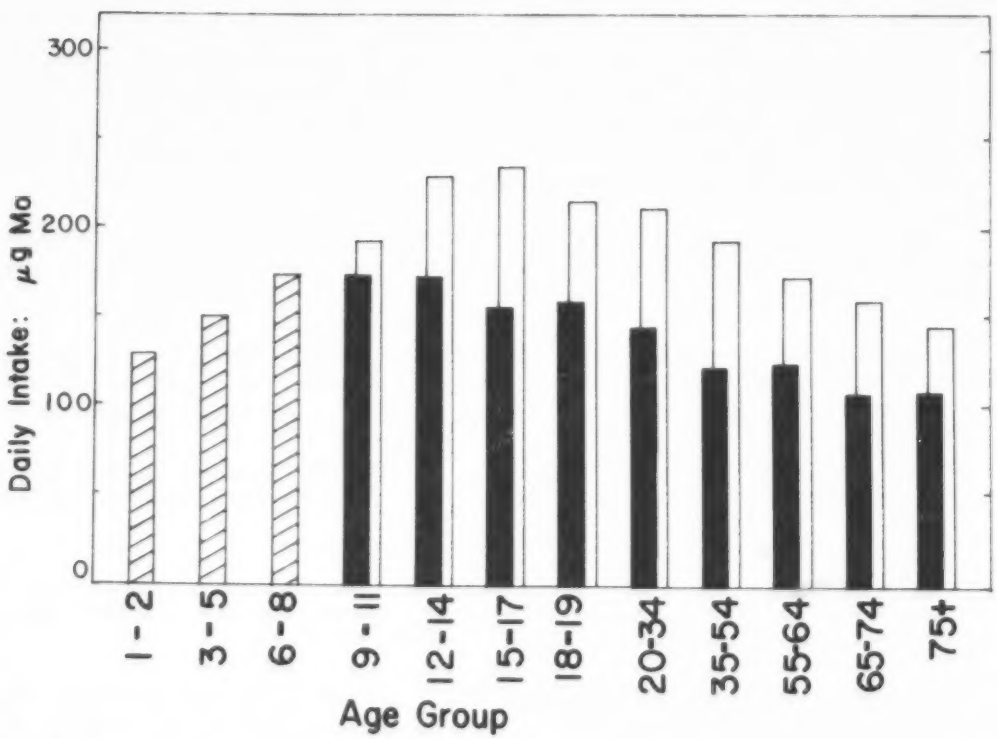

Figure 13. Calculated daily intake of molybdenum according to sex and age groups for the whole United States. Open blocks: males; shaded blocks: females; cross-hatched areas: male and female children less than nine years old.

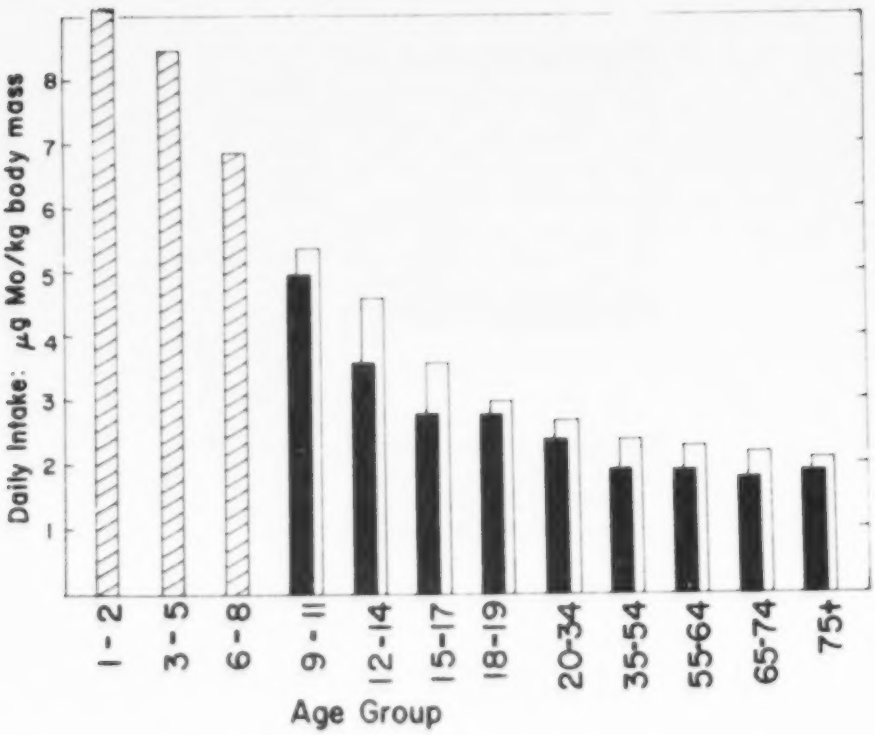

Figure 14. Calculated daily intake of molybdenum per kilogram of body mass, according to sex and age groups for the whole United States. Block legends as in Figure 13.

The proportional contribution of molybdenum to the total dietary intake varies with age because of the differences in the make-up of the diet for these age groups (see "Food" in Section 6 on the molybdenum content of foods.) For example, milk makes up the largest contribution of molybdenum to the diets of children in the United States (approximately 30%), whereas grains and legumes provide most of the molybdenum in adult diets in the United States. Figure 15 illustrates how the contribution of molybdenum to the total dietary intake from foods in 24 categories changes with age.

Molybdenum's essentiality is related to its role in the molybdenum containing metalloenzymes xanthine oxidase, sulfite oxidase, and aldehyde oxidase. The tissue concentration of aldehyde oxidase has been shown to be related to the molybdenum intake in animals, and indirect evidence points to this relationship with the other enzymes (50).

Minimum dietary requirements for molybdenum compatible with satisfactory growth and health cannot be given for any animal species including the human species. Molybdenum deficiency has not been observed under natural conditions with any animal species (82). Although a deficiency of molybdenum in plants

Figure 15. Contribution of major food categories to daily molybdenum intake of male subjects, according to age. The vertical width of each bar is proportional to the fractional portion which the food type contributes to the daily total intake. Examples: Milk contributes 29% of the daily intake of young children and only 10% among 75 year old adults. The contribution from breads increases from 5% to 13% as the individual's age increases. The age scale shown is nonlinear but follows the groups shown in the previous figures. There is a slight decline in bean consumption among 18 to 19 year old males.

has been implicated in the etiology of human illness in South Africa (178), human requirements have not been determined. The information available from balance studies is difficult to assess due to varying amounts of molybdenum in the diets, and inconsistent results with respect to positive and negative balance in subjects with differing dietary intakes of molybdenum (169-171).

Results of some epidemiological and animal studies have indicated that molybdenum exerts some effect in reducing the incidence of dental caries (179-183). In some of the epidemiologic studies, the association with a high molybdenum water supply was not clear; food sources may have been more important contributors of molybdenum. Two separate epidemiologic studies in the United States in California (79) and in Colorado (63) were not able to find evidence

supporting the hypothesis that molybdenum exerts an anti-caries effect. In the California study, dental caries rates were compared in children living in areas with and without molybdenosis problems in cattle. In the Colorado study, dietary intakes of molybdenum among children examined were considered to be comparable due to similar sources of food supply. No consistent association could be found between the use of a high molybdenum content water supply and a reduced incidence of dental caries.

BIOLOGICAL EFFECTS OF MOLYBDENUM IN HUMANS

Deficiency

While molybdenum is considered an essential nutrient, the experimental induction of deficiency states in animals has been difficult and generally required the addition of tungsten to the diets. Only recently have molybdenum responsive syndromes been described in chicks (184) and in goats (185). In humans, nutritional deficiency of molybdenum, per se, has not been described. There has been, however, a report of deficient function of sulfite oxidase, a molybdenum-metallo enzyme, in an infant with neurological abnormalities who died at nine months of age in a decoricate state (186). There were excessive amounts of S-sulfo-L-cysteine, sulfite, and thiosulfate in the patient's urine, concomitant with decreased amounts of inorganic sulfate. Such a pattern could be explained by a block in the conversion of sulfite to sulfate. Since three of the patient's siblings had died in infancy with neurological disorders, the authors postulated that this disorder was an inherited metabolic disease that they called "sulfite oxidase deficiency."

Toxicity

Because of the known toxic effects of molybdenum in animals, there has been concern over possible deleterious health effects of molybdenum exposure or ingestion in humans. Many of the studies on industrial molybdenum exposure were performed in the U.S.S.R., the results of which have been summarized in an excellent critical review by Friberg and others (50).

Early signs of pneumoconiosis were found by Mogilevskaja (cited in ref. 50) in the chest x-rays of a 44 year old woman exposed for five years to molybdenum and MoO_3 in dusts at concentrations ranging from 1 to 3 $mgMo/m^3$, and in a 44 year old man exposed for four years to concentrations varying between 6 to 19 $mgMo/m^3$. Fully developed radiological findings of pneumoconiosis were noted in a 34 year old man after seven years of exposure to the latter concentration, where most of the dust particles were below five microns in diameter. The three subjects, who were among 19 workers in a molybdenum reducing shop, had variable respiratory complaints.

Molybdenum-induced hyperuricemia was reported by Kovalskii and others in 1961 among inhabitants of a molybdenum-rich province in Armenia (2). In this large and complex study, 27% of the adult population of two settlements complained of joint pains, particularly of the knees, the interphalangeal joints, and the metatarso-phalangeal joints of the feet. Articular deformities, erythema, and edema of the joint areas were noted; hepatomegaly was present

and renal and gastro-intestinal disorders were also reported but no specific details given. Hyperuricemia and hyperuricosuria were demonstrated in 17 subjects, in some "healthy" subjects from the same region, and in five controls from a molybdenum-poor province. Elevations of blood molybdenum levels were found in the sick subjects, accompanied by decreases in blood copper concentrations. Urinary excretion of molybdenum did not differ among the "sick" and "healthy" subjects from the Armenian province, but excessive urinary excretion of copper was noted in the sick subjects. The reported control levels of blood and urinary copper raise some doubt as to the accuracy of the measurements in this study, and the control blood molybdenum concentrations also seem high. Serum xanthine oxidase activity was approximately doubled in the sick persons, and was noted to be proportional to increments in blood molybdenum levels. Analysis of the dietary intakes of the inhabitants of the molybdenum-rich province showed daily consumptions of molybdenum to average 10 to 15 mg Mo and that of copper to approximate 5 to 10 mg Cu. Outside the molybdenum-rich province, daily intakes of 1 to 2 mg Mo and 10 to 15 mg Cu were found. While this study lacks adequate control groups and has trace element values which are difficult to interpret, the data presented seems to show increased uric acid levels in blood and urine of the subjects living in the molybdenum-rich province, which may have caused a gouty-type syndrome.

Hyperuricemia is also mentioned in two other studies in the U.S.S.R. accompanied by arthrolgias, and elevations of serum bilirubin, cholestrol and globulin levels among workers in copper-molybdenum plants. No precise laboratory values were presented, hence the validity of these reports is difficult to assess (50). Ecolajan, in 1965, examined 500 workers from a molybdenum and copper mine and compared these workers to a control group of equal size from the general population in the area. The dust levels in the mines were reported to exceed the maximum permissible concentration (in U.S.S.R., MPC = 6 mgMo/m^3) some 10 to 100 fold. Many workers complained of non-specific symptoms such as weakness, fatigue, headaches, irritability, poor appetite, stomach pains, weight loss, irritated skin, dizziness, and tremor (187). The author concluded that molybdenum exposure was accompanied by impairment of central nervous system functions. While the studies in the U.S.S.R. may be difficult to interpret in a scientific manner, they did stimulate further research on the effects of molybdenum on human health, with specific emphasis on uric acid and copper metabolism.

Molybdenum enrichment of sorghum had been noted in India, and Deosthale and Gopalan performed balance studies on four volunteers who were consuming sorghum-based diets that differed only in their molybdenum content (1). The molybdenum concentrations in the two diets provided daily intakes of 160 and 540 µg Mo, respectively, and the authors noted an increase in the mean daily urinary copper excretions from 24 µg to 42 µgCu/24 hours on the higher molybdenum intake.

They then supplemented the sorghum with 1,000 µg Mo as ammonium molybdate to provide a daily intake of 1,540 µgMo/day, which resulted in a further increase in mean urinary copper excretion to 77 µgCu/24 hours. Urinary uric acid excretion remained unchanged at the three levels of molybdenum intake, whereas the mean ± SD plasma copper levels increased from 80.5 ± 11 µgCu/dl on the low molybdenum diet to 113 ± 8.5 µgCu/dl at the high intake. During the

balance studies, daily copper intakes remained constant at 2.4 mgCu/day, as did the fecal copper elimination, which averaged 1.83 mgCu/day. The authors concluded that increases in molybdenum ingestion did not impede copper absorption, but that molybdenum either prevented cellular copper uptake, or mobilized tissue copper stores, or a combination of both mechanisms. The result would be an increase in plasma levels of circulating copper with subsequent increments in urinary copper excretion. They also suggested that changes in uric acid metabolism would occur only at higher intakes of molybdenum.

Human Health Effects and Present Study

Molybdenum contamination of drinking water supplies occurs in some portions of Colorado as a result of seepage from tailing ponds, at molybdenum mines, into downstream creeks and rivers (58). In our study levels of molybdenum as high as 400 µgMo/L were found in the water supply of the city of Golden water source which received effluent from the tailings ponds of the Urad mine. Such high levels were a cause of concern and the U.S. Environmental Protection Agency sponsored an interdisciplinary study by this group of the biological effects of molybdenum in humans, with the particular aim of recommending an acceptable level of molybdenum for drinking water in the United States. To accomplish this purpose, it was necessary to first determine normal concentrations of molybdenum in plasma and urine, and biological samples were collected from subjects in the Denver area, where the water content of molybdenum does not generally exceed 50 µgMo/L. Since molybdenum was known to affect copper and uric acid metabolism, the blood samples were also assayed for ceruloplasmin (a cuproprotein that normally contains 90% of circulating plasma copper) and/or copper content, and serum uric acid levels were determined. The urinary levels of copper and creatinine were also measured, the latter being useful to judge the completeness of a timed urinary collection, and for analysis of uric acid to creatinine and molybdenum to creatinine ratios. The levels of serum glumatic-oxalecetic transaminase (SGOT) were measured as an indicator of hepatic function and serum creatinine and/or blood urea nitrogen (BUN) were also assayed as indicators of renal function. Medical histories were obtained at the time of sample collections, primarily on male subjects. The known effect of female estrogenic hormones on plasma copper and ceruloplasmin levels could mask any molybdenum-induced changes. The results of the biochemical assays performed on these subjects were then compared to those of subjects with high molybdenum exposure. Such exposure arose from the presence of the metal in drinking water or in industrial settings

One study of industrial exposure was performed at a roasting plant in Denver where molybdenum sulfide is converted to molybdenum oxides (45). Twenty-five workers at the plant responded to a general medical questionnaire and provided a blood sample. Among these workers seven had no complaints; six had incurred an upper respiratory infection in the preceding two weeks; six complained of both joint pains and backaches; another four complained of joint pains; and another four of backaches alone. Diarrhea was mentioned by five workers, headaches by four, and non-specific hair or skin changes by eight of the workers. In general, the complaints were not very specific and there were no complaints of gout or renal stones among the workers. Pulmonary function tests (forced vital capacity and forced expiratory volume/second) were normal

in 22 of the 25 workers, but evidence for mild obstructive lung disease was present in three subjects aged 32, 52, and 59 years.

Total dust samples were collected in the roaster area and an eight-hour time weighted average (TWA) of exposure to molybdenum was calculated to be 9.47 mgMo/m^3. X-ray diffraction studies of the dust showed it to be mainly composed of molybdic oxide (MoO_3) and other soluble oxides of molybdenum. Respirable dust samples were collected with a system that limited entry to particles equal or smaller than 10 microns in diameter. The molybdenum concentration of respirable dust at the base of the roaster was 1.02 mgMo/m^3 of air. Since a worker breathes an average 10 m^3 of air per eight-hour shift and the particle size was sufficiently small to allow penetration into at least the upper airways, the minimum daily body burden of molybdenum was calculated to be 10.2 mg Mo.

The biochemical assays performed on the workers were compared to levels obtained from a control group, which at that time, consisted of research personnel at the University of Colorado Medical Center. The complete blood counts were normal but the industrial workers demonstrated significant increases in serum ceruloplasmin and milder increases in serum uric acid levels (Table 17).

TABLE 17. SERUM CERULOPLASMIN AND URIC ACID CONCENTRATIONS IN MOLYBDENUM FACTORY WORKERS AND IN CONTROL MALES (MEAN ± SE)

	Number	Ceruloplasmin (mg/dl)	Uric acid (mg/dl)
Workers	(25)	50.47 ± 1.38	5.90 ± 0.24
Controls	(24)	30.50 ± 1.33	5.01 ± 0.25
p-value		<0.005	<0.025

Fourteen of the 24 control subjects had plasma molybdenum concentrations less than 5 µgMo/L which was the lower limit of detection. In the 10 remaining control subjects the plasma molybdenum levels ranged from 5 to 34 µgMo/L. In contrast, among the factory workers, plasma molybdenum concentrations ranged from 9 to 365 µgMo/L, and there were no samples below 5 µgMo/L. Only 10 of the 25 samples were within the range of the control; levels of plasma molybdenum from 35 to 100 µgMo/L were present in seven subjects, from 101 to 299 µg Mo/L in five and greater than 300 µgMo/L in three. These differences were highly significant ($p<0.005$) by the Wilcoxon Rank Sum Test (see Fig. 16).

Fourteen of the industrial workers provided urine collections which unfortunately were incomplete, as determined by calculation of the daily urinary creatinine excretions. Urinary molybdenum content was hence expressed in µg/L and for control subjects the range was from 20 to 230 µgMo/L. In the samples from the workers only two were within the normal range. Levels from 450 to 1,000 µgMo/L were present in seven subjects, from 1,000 to 3,000 µgMo/L in

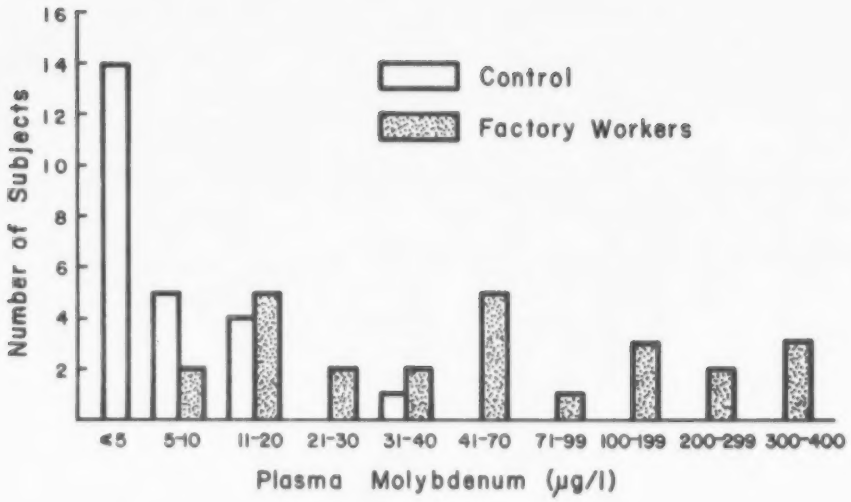

Figure 16. Plasma molybdenum concentrations of control subjects and of workers in a molybdenum smelter in Denver.

four, and 11,000 µgMo/L in one (see Fig. 17). The urinary copper excretion was normal (less than 50 µgCu/L) in 13 of the 14 subjects, but hypercupruria (347 µgCu/L) was present in the last sample. The urinary uric acid/creatinine ratios were normal (i.e., less than 0.75) in the 14 workers.

This pilot investigation demonstrated absorption of molybdenum from dust particles with subsequent excretion by the kidneys. It could not be determined whether molybdenum was absorbed through the lungs or by the gastrointestinal tract after swallowing of secretions. The wide range of plasma molybdenum levels in the workers may be explained by the time of day when the samples were taken. For some workers, the samples were taken prior to the eight-hour shift, others in the middle of the work day, and some at the end of the work day.

The increments in mean serum uric acid levels of the Denver factory workers were not as marked as those described by Kovalskii and others (2) and hyperuricosuria was not found in our study. The increases in mean serum ceruloplasmin levels among Denver factory workers were paralleled by proportional changes in plasma copper content, and hence are in discordance with the Russian author's findings of decreased levels of plasma copper. Elevation of serum ceruloplasmin could be expected, however, according to Deosthale and Gopalan's hypothesis (188), if molybdenum exposure leads to mobilization of tissue copper stores within the hepatocyte, with subsequent synthesis of ceruloplasmin to prevent intracellular copper toxicity (164).

This pilot investigation in factory workers raised the question of whether serum ceruloplasmin levels could be used as an indicator of excessive molybdenum exposure. While the mean uric acid levels were also significantly

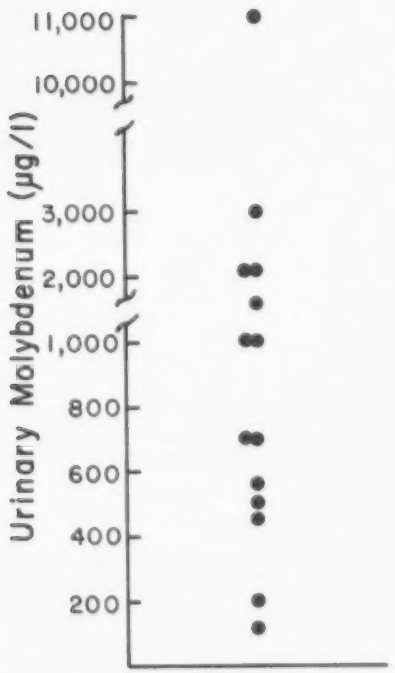

Figure 17. Urinary concentrations of 14 workers in a molybdenum smelter.

higher than in the control group, the mean value still remained within the normal range.

Industrial exposure to molybdenum dusts also occurs in the mining industries, and we collected samples in two groups of workers at molybdenum mines in Colorado and New Mexico. Dust samples were taken at various levels in a molybdenum mine by Rafael Moure who measured dust levels in various sections of the mine. The levels ranged from 0.32 to 1.32 mgMo/m^3, eight-hour time weighted average (TWA) of respirable dust and were hence much lower than in the factory setting. These values were compounded from 76 personal dust samples collected during the investigation. Fourteen of the samples were analyzed for MoS_2 content which varied from 0.2 to 0.49 µgMo/m^3. The estimated net daily exposure ranged from 1 to 368 µgMo/day. Blood samples were obtained from 16 workers at the Climax mine, most of whom were involved with the milling operation. The mean ± SE (standard error) ceruloplasmin level in these subjects was 42.31 ± 2.54 mg/dl and the mean plasma copper levels were 129.38 ± 5.47 µgCu/dl. Both mean levels were above control values for males. The mean serum uric acid concentration was 6.5 ± 0.40 mg/dl for 15 subjects, one having been excluded because he was taking medications known to affect serum uric acid concentrations. Plasma molybdenum concentrations were below 5 µgMo/L in 12 of 15 samples assayed and were 6, 9, and 28 µgMo/L, respectively, in the remaining three samples. Urine collections were obtained in six of these workers and the urinary molybdenum concentrations ranged from 26 to 85 µgMo/L

with the urinary copper levels varying between 5 to 12 µgCu/L. Calculations of the diurnal creatinine excretion showed low values in four of the samples. In the remaining two, which appeared adequate, the 24-hour urinary molybdenum excretion was 41 µg and 48 µg, respectively. Urine uric acid to creatinine ratios were normal.

Blood samples were also obtained from 18 miners at the Questa mine in New Mexico. The mean ± SE serum ceruloplasmin for 18 Questa miners was 40.94 ± 1.66 µg/dl and the mean serum uric acid was 6.09 ± 0.27 mg/dl for 15 subjects. The uric acid levels of three miners were not included in the calculation because one had long-standing gout (serum uric acid: 10.1 mg/dl) and two others were taking antihypertensive medications (serum uric acid: 8.8 and 10.2 mg/dl). Plasma molybdenum levels were below 5 µgMo/L in 12 of the 18 samples, and ranged from 6 to 18 µgMo/L in the remaining six samples. Because of technical difficulties it was not possible to collect timed urine specimens in the Questa miners, but aliquots of urine were provided by 11 miners. The uric acid to creatinine ratios were normal. The urinary molybdenum concentrations varied from 20 to 74 µgMo/L and the molybdenum to creatinine ratios (urinary molybdenum in µg/L/urinary creatinine in mg/L) ranged from 0.02 to 0.05.

The results obtained in the miners differed from those of the factory workers in various aspects. The plasma and urinary concentrations of molybdenum were uniformly low in the miners, which might be expected, since the subjects were mainly exposed to molybdenite (MoS_2), a compound known to be relatively insoluble. In contrast, the serum uric acid levels were higher among the miners, but this hyperuricemia could be a consequence of polycythemia secondary to altitude. Leadville, where the Climax miners reside, is situated at 10,000 feet above sea level, and while the altitude of the Questa village approximates 7,000 feet, the actual mine location is around 9,000 feet.

The mean serum ceruloplasmin levels in the miners were higher than in the Denver control subjects, but did not attain the levels demonstrated by the workers at the molybdenum roasting plant. Since no evidence for excessive molybdenum exposure was present, the question of whether altitude might also affect serum ceruloplasmin levels was entertained and a decision was made to collect samples from subjects living at high altitudes and not involved in molybdenum mining operations. The necessity for caution in the interpretation of serum ceruloplasmin and uric acid levels, as indicators of molybdenum exposure, became evident with these studies, while at the same time, the reliability of plasma and urinary molybdenum values increased.

In addition to these studies of industrial exposure, molybdenum is present, as previously mentioned, in some drinking water supplies of communities in Colorado. The highest quantities of molybdenum used to be found in the water of the city of Golden where levels of 300 to 400 µgMo/L had, on occasions, been measured. However, cessation of the operations at the Urad molybdenum mine resulted in decreased contamination of the Clear Creek river, and the levels of molybdenum in Golden water supplies have been decreasing since 1974. To determine whether molybdenum in water was accompanied by any health effects or changes in biochemical indicators, we studied various populations whose water supplies differed in molybdenum content. As controls a group of subjects were selected from the Denver area, where the molybdenum in water

fluctuates from 1 to 50 µgMo/L. Blood samples were collected on 42 male subjects whose age ranged from 19 to 46 years. The mean ± SE serum ceruloplasmin level for the group was 30.41 ± 1.03 µg/dl and the mean serum uric acid was 5.34 ± 0.20 µg/dl, both levels being in accordance with published normal values. Plasma molybdenum concentrations varied from less than 5 µgMo/L in 18 subjects to 34 µgMo/L. Fourteen subjects had levels from 5 to 9 µgMo/L; levels from 10 to 19 µgMo/L were found in seven persons, from 20 to 29 µgMo/L in one, and the two remaining subjects had plasma molybdenum concentrations of 33 and 34 µgMo/L, respectively (see Fig. 18). Red blood cell molybdenum content varied between less than 5 to 38 µgMo/L. Twenty-four hour urine collections were performed on 14 controls, for which complete results are available on 12 subjects. The urinary uric acid to creatinine ratios were normal in all subjects. The molybdenum concentration varied from 120 to 230 µgMo/L, with the mean 24-hour excretion of molybdenum being 87.25 ± 18.02 µg (SE). Urinary copper concentrations varied from 4 to 25 µgCu/L, the mean daily copper excretion being 12.70 ± 1.50 µgCu/24 hours. Urinary molybdenum to creatinine ratios ranged from 0.01 to 0.11.

Since the levels of molybdenum in the water of the city of Golden were decreasing, a sample collection was organized in March 1975, at which time the water levels of molybdenum approximated 200 µgMo/L. Blood samples were drawn on 13 university students at the School of Mines and the mean ± SE serum ceruloplasmin was 40.31 ± 2.55 mg/dl, a level significantly higher ($p<0.005$) than for the control group. The mean serum uric acid was 4.35 ± 0.46 mg/dl, significantly lower than for the control group. The mean daily molybdenum excretion in four 24-hour urines was 186 ± 34 µg, also significantly higher than for the control group ($p<0.01$), and the mean daily urinary excretion of copper was 11.63 ± 4.02 µgCu; the latter value did not differ from the control group.

Another sample collection was organized in the Golden area in September 1977, by which time the water content of molybdenum had dropped to approximately 40 µgMo/L. Blood samples were collected again in 13 students and a comparison of the serum ceruloplasmin and uric acid levels and of the urinary copper and molybdenum excretions is shown in Table 18.

The decrease in molybdenum water content in the Golden area was accompanied by normalization of the serum ceruloplasmin levels and by a decrease in the mean daily urinary molybdenum excretion. The increase in mean serum uric acid levels demonstrated by the students in 1977 has to be interpreted with caution, since at the time of collection, it was evident that many of the students had been drinking beer, and alcohol is known to increase serum uric acid levels through inhibition of renal secretory mechanisms. The students were asked to estimate their daily intake of all beverages and the estimated quantities varied between two to three liters per day, of which approximately one-half were water or beverages made from water. Hence, the daily contribution of molybdenum water in 1975 would have approximated 300 µg. Calculations of the molybdenum intake from foods show that for 20 year old male subjects the daily ingestion approximates 210 µg, so the total molybdenum ingested in food and beverages in 1975 would have reached 500 µg daily. At such a level of intake, the serum uric acid levels were not affected, serum ceruloplasmin concentrations increased, and plasma molybdenum levels were within the normal

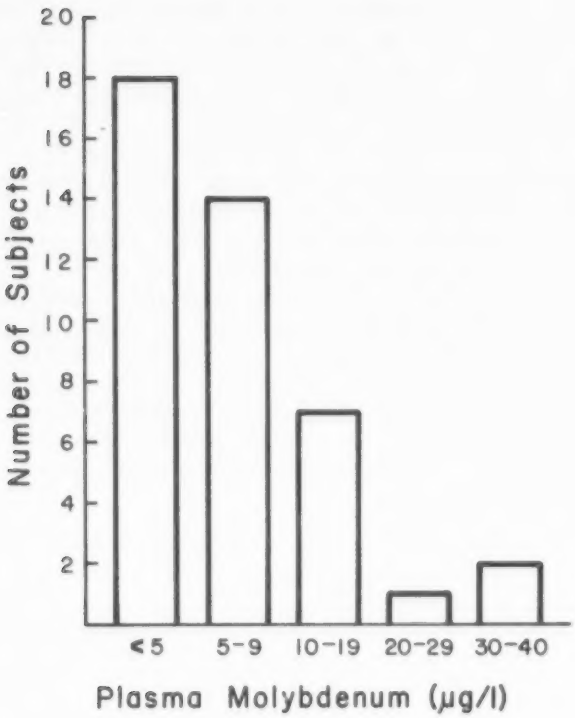

Figure 18. Histograms of plasma molybdenum concentrations in 42 normal subjects from the Denver area.

TABLE 18. COMPARISON OF SERUM CERULOPLASMIN AND URIC ACID LEVELS AND OF THE URINARY EXCRETIONS OF MOLYBDENUM AND COPPER IN 1975 AND 1977 IN THE GOLDEN AREA (MEAN ± SE)

	(N)*	1975	(N)	1977	p-value
Serum ceruloplasmin (mg/dl)	(13)	40.31 ± 2.55	(13)	34.15 ± 1.74	<0.05
Uric acid (mg/dl)	(13)	4.35 ± 0.46	(13)	5.88 ± 0.27	<0.005
Urinary molybdenum (µg/24 hrs)	(4)	186 ± 34	(8)	88 ± 10	<0.005
Urinary copper (µg/24 hrs)	(4)	11.63 ± 4.02	(8)	14.20 ± 1.62	N.S.

*Number of subjects

range. Twelve of the 13 subjects in 1975 had plasma molybdenum concentrations less than 5 µgMo/L. The urinary copper excretion did not seem to be affected by the elevated levels of molybdenum in the water. In contrast, the urinary excretion of molybdenum was higher, thus indicating renal clearance of the excess molybdenum ingested.

Another occasion to compare two populations with different water concentrations of molybdenum occurred in the metropolitan Denver area. The molybdenum concentrations in water of a Denver suburb fluctuated in 1977 between 80 to 100 µgMo/L while the levels in the city of Denver were generally less than 40 µgMo/L. Blood and urine samples were collected from 13 workers at the suburban municipal water treatment plant and comparison was performed with the results obtained from samples collected from 16 workers at a Denver area water treatment plant (Table 19). The plasma molybdenum levels were within the normal range in the workers from both area treatment plants. Serum ceruloplasmin levels were similar in both groups, and there were no significant differences in the daily urinary excretion of copper, which was higher in the workers from the suburban treatment plant.

TABLE 19. COMPARISON OF SERUM CERULOPLASMIN AND URIC ACID LEVELS AND OF THE URINARY EXCRETIONS OF MOLYBDENUM AND COPPER IN TWO GROUPS OF WORKERS AT WATER TREATMENT PLANTS (MEAN ± SE)

	(N)	Denver	(N)	Suburban area	p-value
Serum ceruloplasmin (mg/dl)	(16)	38.69 ± 1.59	(13)	38.46 ± 0.32	N.S.
Serum uric acid (mg/dl)	(16)	5.74 ± 0.46	(13)	6.25 ± 0.32	N.S.
Urinary molybdenum (µg/24 hrs)	(9)	88 ± 19	(9)	97 ± 10	N.S.
Urinary copper (µg/24 hrs)	(8)	12.63 ± 1.65	(10)	22.91 ± 1.90	<0.005

An occasion to compare another group of subjects with different concentrations of molybdenum in water arose from a unique situation existing in neighboring communities in the Summit County area of the Rocky Mountains. In the water supplies of Breckenridge and Dillon, only traces of molybdenum have been found whereas in Frisco and Silverthorne, levels between 100 to 400 µgMo/L have been repeatedly measured by this group. Furthermore, these communities are situated at an altitude of 9,000 feet and hence it is possible to determine whether altitude caused the changes in serum uric acid and/or ceruloplasmin levels found in the mines.

Blood and urine samples were collected from 28 male and female inhabitants who had resided in the area for at least two years. Nine male and eight

female subjects were from the high-molybdenum areas, and five males and six females served as controls from the low-molybdenum areas of Breckenridge and Dillon. The results of biochemical assays performed on the male subjects are shown in Table 20; no significant differences were found. The plasma molybdenum levels in subjects from both sexes and from both drinking water areas were within the normal range. Corresponding results of biochemical tests in the females are shown in Table 21, where, interestingly, the female residents from the high-molybdenum area excreted significantly more molybdenum in the urine.

TABLE 20. COMPARISON OF SERUM CERULOPLASMIN AND URIC ACID LEVELS AND OF THE URINARY MOLYBDENUM AND COPPER EXCRETIONS IN RESIDENTS OF SUMMIT COUNTY (MEAN ± SE); MALES ONLY

	(N)	Low molybdenum in water (10 µg/L)	(N)	High molybdenum in water (140 µg/L)	p-value
Serum ceruloplasmin (mg/dl)	(5)	41.00 ± 0.71	(9)	37.22 ± 2.58	N.S.
Serum uric acid (mg/dl)	(5)	6.58 ± 0.32	(9)	6.68 ± 0.33	N.S.
Urinary molybdenum (µg/24 hrs)	(4)	72 ± 14	(6)	112 ± 32	N.S.
Urinary copper (µg/24 hrs)	(4)	14.95 ± 1.59	(6)	23.96 ± 6.30	N.S.

TABLE 21. COMPARISON OF SERUM CERULOPLASMIN AND URIC ACID LEVELS AND OF THE URINARY MOLYBDENUM AND COPPER EXCRETIONS IN RESIDENTS OF SUMMIT COUNTY (MEAN ± SE); FEMALES ONLY

	(N)	Low molybdenum in water (10 µg/L)	(N)	High molybdenum in water (140 µg/L)	p-value
Serum ceruloplasmin (mg/dl)	(6)	49.33 ± 4.03	(7)	49.57 ± 3.41	N.S.
Serum uric acid (mg/dl)	(6)	5.10 ± 0.17	(8)	4.63 ± 0.28	N.S.
Urinary molybdenum (µg/24 hrs)	(5)	57 ± 9	(8)	100 ± 14	0.025
Uninary copper (µg/24 hrs)	(5)	8.71 ± 1.28	(8)	22.61 ± 7.24	N.S.

An estrogen effect on serum ceruloplasmin concentrations is possible, as is the protective effect of female hormones on uric acid levels. Also of interest was the comparison between the serum levels of ceruloplasmin and uric acid obtained in Summit County to those obtained in the Denver area control groups.

Significant elevations of serum ceruloplasmin ($p<0.005$) and of uric acid ($p<0.025$) were noted in the male residents of the low-molybdenum Breckenridge area when compared to the Denver subjects. Similar significant differences were present for the high-molybdenum Frisco area residents, when compared to Denver controls. Furthermore, the urinary excretion of copper in male Frisco residents was significantly higher than in the Denver subjects (Table 22).

TABLE 22. COMPARISON OF BIOCHEMICAL ASSAYS IN MALE SUBJECTS FROM THE DENVER, BRECKENRIDGE, AND FRISCO AREAS (MEAN ± SE)

Water molybdenum content	Denver (N)	Denver 0-50 µgMo/L	Breckenridge (N)	Breckenridge 20 µgMo/L	Frisco (N)	Frisco 150-200 µgMo/L
Serum ceruloplasmin (mg/dl)	(42)	30.41±1.03	(3)	41.00±0.71*	(9)	37.22±2.58*
Serum uric acid (mg/dl)	(42)	5.34±0.20	(5)	6.58±0.32*	(9)	6.68±0.33*
Urinary molybdenum (µg/24 hrs)	(12)	87±18	(4)	72±14	(6)	112±32
Urinary copper (µg/24 hrs)	(12)	12.70±1.50	(4)	14.95±1.59	(6)	23.96±6.36*

*Significantly higher than for the Denver control values ($p<0.025$ or less)

The results of these different investigations point out the difficulties of assessing molybdenum exposure with indirect indicators, i.e., serum ceruloplasmin and uric acid levels. While serum ceruloplasmin levels seemed to be affected by molybdenum exposure in the molybdenum factory workers and in the 1975 Golden area students, ceruloplasmin levels were also affected by gender, altitude, and occupation. Similarly, the potential effects of molybdenum on serum uric acid values could be masked by age, gender, alcohol intake, and altitude. With so many variables, it became evident that the best indicators of molybdenum exposure were the plasma and urinary levels of the metal. It was encouraging to find levels of plasma molybdenum within the normal range (5 to 34 µgMo/L) among subjects who were consuming water with 200 µgMo/L. Increased ingestion was generally accompanied by increased urinary excretion of molybdenum (see Fig. 19), a finding noted in the Golden area students, the residents of Summit County, and the molybdenum factory workers. In the latter, workers where molybdenum exposure was far greater than those in any of the other situations, the plasma levels and the urinary molybdenum to creatinine ratios were elevated. Renal clearance of excess molybdenum thus seems to function as a protective homeostatic mechanism in humans as it does in animals. Excessive

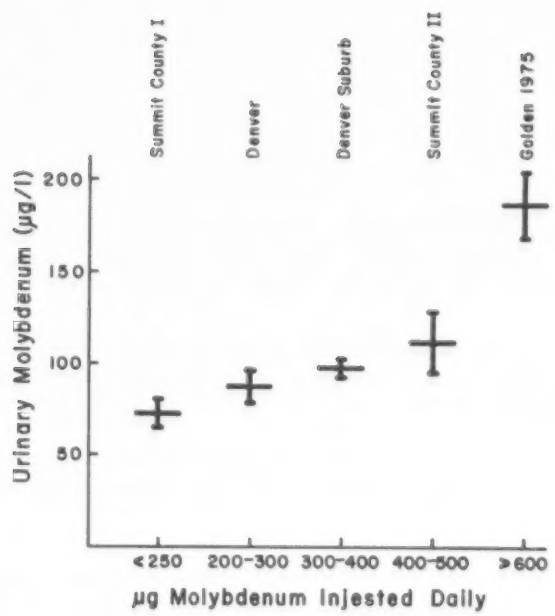

Figure 19. Comparison of the daily urinary molybdenum excretion (mean ± SE) in male subjects with different daily intakes from food and water.

Summit County I --- Breckenridge
Summit County II -- Frisco
Golden 1975 ------- Students in 1975

urinary copper excretion was not demonstrated in the present studies, even when significant differences in copper excretion were shown. Nor was increased urinary uric acid excretion found in the various samplings performed. Since no biochemical changes were found in subjects consuming water containing up to 50 µgMo/L, it can be assumed that such a level does not cause any adverse biochemical or health effects. Biochemical changes were seen in some subjects at 100 µgMo/L. The argument is further corroborated by the observations in the Golden area students where the changes in molybdenum water concentrations were accompanied by normalization of serum ceruloplasmin levels and of urinary molybdenum excretion.

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APPENDIX A

SUPPLEMENTARY CALCULATION OF GUIDELINE

The guideline of 50 µgMo/L for human drinking water is based upon animal and human studies described in the preceding sections. In order to "check" this guideline, we have performed the following calculations based upon dietary intakes. While the validity of the initial assumptions necessary for the calculations may be argued, we believe that the calculations provide a useful order of magnitude check on the guideline.

Early humans probably did not have a "balanced" diet since availability of game and vegetable stuffs was variable. The hunter-gatherers probably existed on a vegetarian diet for short periods of time and on a meat diet at other times. These short-term fluctuations of food intake probably resulted in short-term deficiencies and excesses of trace elements (see Table 10, Section 6). Some level of adaptability to variable intakes probably still exists in humans today. Modern food distribution practices and dietary habits tend to minimize the fluctuations of trace element intakes. However, some differences in food consumption exist between the sexes and among the various age groups. Socioeconomic factors and dietary preferences also lead to different intakes. Since there is no evidence that severe deficiencies or excesses occur in the United States' population, we may assume that the biological and socioeconomic differences are within the adaptability of humans.

Table A-1 shows the variability of dietary intake from foods for several biological and socioeconomic groups. These results were obtained by calculating the maximum difference in daily molybdenum intake for the various groups. (Percentages are based on the average intake of the two groups being compared. See Figures 13 and 14, Section 8.) These calculations show that the maximum intake difference observed between the sexes or with age is about 50%. Socioeconomic variables produce differences less than or equal to biological variables. If we assume that the observed biologically-based intake variation of 50% is the maximum perturbation that may be tolerated without observing effects, then it is possible to calculate the concentration of molybdenum in water which would produce an equivalent increase.

Using the calculated dietary intakes (Figure 13, Section 8), and the availability data on consumption of water-based beverages (coffee, tea, soft drinks from reference 77) one can calculate the molybdenum concentration in water which would produce a 50% increase in dietary intake.

The results of these calculations for the average individual in each age and sex category are shown in Figure A-1. The apparently high concentrations

TABLE A-1. VARIABILITY OF DAILY MOLYBDENUM INTAKE

Differences		Maximum % variation
Biological (adults only)	Age (males)	47
	Age (females)	47
	Sex (males* vs females)	42
	Age (μg/day/kg body weight)	47
Socioeconomic	Income (low* vs high)	52
	Urbanization (urban vs rural*)	15
	Geographic (North U.S. vs South U.S.*)	6
Other	Meat vs vegetarian*†	58

*Group with higher molybdenum intake.

†Vegetarian diet estimated by substituting a proportionate increase of all non-meat components for meats (estimate only)

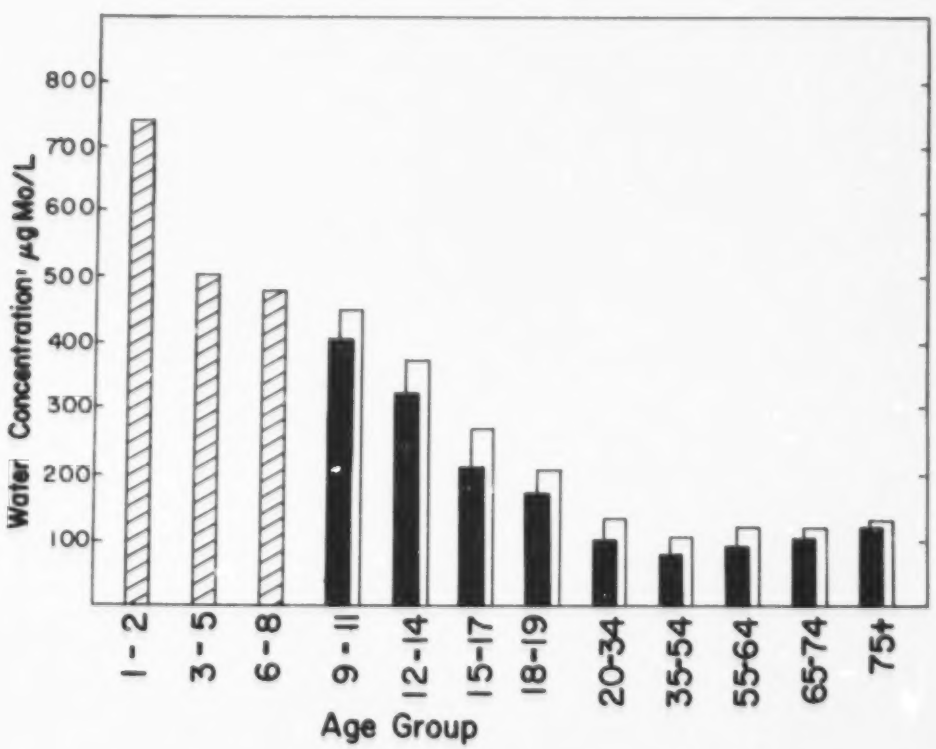

Figure A-1. Molybdenum concentration of water required to produce a 50% increase in daily intake through water-based beverages.

of molybdenum in water permissible among younger individuals is mainly due to the small amount of water-based beverages consumed by this group. However, among adults the predicted molybdenum concentration which would produce a 50% increase in daily intake approaches 100 µgMo/L. If we assume that water intake is about equal to the water-based beverage intake*, then the "permissible" water concentration would be about 50 µgMo/L. This estimate is the same as the guideline based upon the studies described in previous sections. The agreement between the calculated estimate and the guideline may be fortuituous but it serves as a useful order of magnitude check.

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* The maximum water-based beverage intake of 0.85 liters occurs among males aged 35 to 54. Doubling this amount leads to a value close to the 2.0 liters typically used by the U.S. Environmental Protection Agency.

APPENDIX B

ANALYSIS FOR MOLYBDENUM, SPECTROPHOTOMETRIC METHOD

Thiocyanate has been used extensively in the colorimetric analysis of molybdenum (13-16). The conventional analytical method has been modified slightly to suit our needs. Water samples can be routinely analyzed with precision limits of about ±5 µg/L (without prior concentration steps). This procedure is outlined below.

ANALYSIS OF WATERS FOR MOLYBDENUM

1. Pipet 50 mL of the unknown or standards into a 250 mL separatory funnel. (A blank should also be carried through this procedure. The standards which we use usually range from 50 to 500 µg/L. Typically we run four: 50, 100, 300, 500 µg/L.)

2. Add 2 mL of concentrated HCl to the samples. The acid is required to prepare the solution for the formation of the thiocyanate complexation reaction. This reaction requires that the final solution be about 1 M in acid.

3. Add approximately 0.2 g of sodium tartrate. This "ties up" any tungstate which may be present. It would interfere with the analysis for molybdenum since tungsten also forms a colored complex (yellow) with thiocyanate.

4. Add 0.5 mL of 1% ferrous ammonium sulfate. Prepare this solution by weighing out 1 g of the reagent grade $Fe(NH_4SO_4)_2$. Dissolve this salt in 100 mL of deionized water and 1 mL of concentrated sulfuric acid.

5. Add 3.0 mL of 10% KCNS. Prepare this solution fresh <u>daily</u> by weighing 5.0 g of the salt and dissolving it in 45 mL of deionized water.

6. Allow the solution to stand for about 15 minutes. This step is very important. Full color will not develop if time is not given for the complexation reaction to be completed. The solution will be pink, orange, or red.

7. Add 9.0 mL of 10% stannous chloride solution. Prepare this by weighing out 100 g of the salt and dissolving it in 125 mL of concentrated HCl. Heat the mixture until all of the salt dissolves.

The solution should be clear. Slowly add this solution to 900 mL of deionized water. Store this solution in a bottle which contains a few pieces of pure tin metal.

8. Allow the solution to stand for at least 15 minutes. The pink or red color should disappear. Only a pale amber color should remain. This is the thiocyanate-molybdenum complex.

9. Add 10.0 mL of iso-amyl alcohol to the separatory funnel. Shake the funnel vigorously until the color leaves the aqueous phase and enters the organic phase. Thirty to sixty seconds should be sufficient.

10. Allow the phases to separate (about 15 minutes). Drain off the aqueous layer and discard it. Be sure to get all of the water out. If necessary, drain off a little of the organic layer to "flush" any water out of the stopcock bore.

11. Drain the colored organic layer into a test tube. Add a spatula full of granular anhydrous sodium sulfate to the solution and allow it to stand about 30 minutes. This drying step helps to remove any turbidity which could interfere with the spectrophotometric readings.

12. Read the absorbance at 465 nm versus the blank. Prepare a Beer's Law plot of absorbance versus concentration using the standards. Use the plot to determine the concentrations of the unknowns.

In this lab we usually do a linear least squares regression analysis on the standard points. From the equation of the line we can calculate the concentration of the unknowns.

The conventional thiocyanate procedure has been shortened to permit more rapid analysis of samples. The increased speed of the analysis has been gained at the expense of some precision. The standard error of estimate is typically ±15 µg/L. This method is referred to as the semi-quantitative procedure. Most natural waters and aqueous samples can be analyzed by this procedure.

More Rapid Procedure

This procedure is used for the rapid processing of large numbers of samples. Up to fifty samples may be analyzed at a time. If the samples are higher than 1 mg/L the solution should be diluted to fall in the range of the standards. This procedure uses 30 mL test tubes. All of the steps and waiting times are the same as in the method described above. A standard deviation of about 15 µg/L can be expected when this method is used.

The following are the changes in reagent quantities to be used:

1. 10 mL samples and standards.

2. 0.5 mL concentrated HCl.

3. 0.05 g tartrate (exact amount not critical).

4. 0.1 mL of 1% ferrous ammonium sulfate solution.

5. 0.5 mL of 10% potassium thiocyanate solution.

6. 2.0 mL of stannous chloride solution.

7. Extract into 4.0 mL of iso-amyl alcohol.

8. Centrifuge the test tubes and contents at 2,000 rpm for about five minutes.

9. Draw off the colored organic phase by using an 8" Pasteur pipet.

10. Dry the extract over anhydrous sodium sulfate. (Omit if AA is used on the organic extract!)

11. Allow the extracts to stand for about one-half hour. Read the absorbance at 465 nm. Use 1 cm absorbtion cells.

APPENDIX C

ANALYSIS FOR MOLYBDENUM, ATOMIC ABSORPTION SPECTROPHOTOMETRY (AAS)

Molybdenum analysis by flame AAS is susceptible to many interferences. Direct aspiration of aqueous samples is not recommended because of these interferences. In addition, the method generally affords a detection limit (signal to noise ratio of 2.0) of about 50 µgMo/L. Therefore the method has insufficient sensitivity and precision to adequately determine the "contamination" of most waters.

Acceptable results can be obtained using flame AAS by preconcentrating the samples prior to analysis. The analyte should also be separated from potential interferants. Both of these requirements can be accomplished by using a complexation-solvent extraction procedure. Several publications describing such procedures are referenced in Section 4 of the text.

In this laboratory we have successfully used the following procedure. It consists of the complexation and solvent extraction technique used for the spectrophotometric determination of molybdenum described in the preceding pages. However, in this modification molybdenum detection is accomplished by aspirating the organic solvent containing the analyte into a nitrous oxide-acetylene flame. Lower detection limits and higher sensitivity are achieved by using greater preconcentration factors (sample volume to solvent volume ratios). In addition, the sensitivity is improved when organic solvents are used in flame analysis of refractory metals such as molybdenum.

Sample aliquots of 10 to 15 mL are taken for analysis. (This sample volume permits a detection limit of about 5 µgMo/L.) The following method is analogous to the solvent extraction technique described in the preceding pages.

1. Add 0.5 g sodium tartrate and stir.
2. Add 0.1 mL 1% ferrous ammonium sulfate solution.
3. Add 0.5 mL 10% potassium thiocyanate solution; stir; wait 15 minutes.
4. Add 2.0 mL 10% stannous chloride solution; stir; wait 15 minutes.
5. Add 2.0 mL of i-amyl alcohol to the samples and 4.0 mL to the standards; shake; allow 15 minutes for layers to separate; centrifuge for about 5 minutes.

6. Analyze the organic extract for molybdenum using flame atomic absorption.

AA parameters--Perkin-Elmer 360

Lamp wavelength--313.3 nm
Lamp current--30 ma
Flame N_2O-acetylene--fuel rich, 5 cm single slot nitrous oxide head
Scale expansion x 25
Aspiration rate--the nebulizer system is slightly adjusted for the alcohol to aspirate moderately slow

The standards are 50, 100, 200, and 300 µgMo/L. An iso-amyl alcohol blank is used. The common practice in this laboratory is to include two to three of each standard, depending on the size of the run, to insure that there is surplus to monitor the calibration of the instrument.

The absorption is determined by using several six second time averages for each sample and standard. A Beckman 10" strip chart recorder is used to display the time integrals. After analysis the peak heights are measured and the concentrations are calculated.

Flameless AAS or electrothermal atomization techniques which use a heated graphite furnace or cup may also be used for molybdenum analysis. The graphite furnace technique permits molybdenum determination at the ng level with small sample volumes. This technique is, however, prone to matrix interferences when used for molybdenum analysis. The boiling point of molybdenum metal is about 4,800°C. This is about 2,000°C above the maximum temperature obtained in the graphite furnace. This indicates that molybdenum atomization can only take place by a mechanism which includes the formation of a compound which is volatile at 2,800°C. This compound then can dissociate in the vapor phase to release molybdenum atoms. Any ions or compounds which affect the complicated atomization mechanism will alter the sensitivity of the method. This crucial fact is the primary reason for the extreme matrix sensitivity of the method.

The principal interference encountered in this study results from the simultaneous presence of two ionic species, an alkali metal ion such as potassium or sodium and the sulfate ion. Neither the cation or anion produce significant interference when they are experimentally added with other counter ions. A sample containing 50 µgMo/L will appear to contain 30 µgMo/L when sulfate (in the presence of sodium) is present at 1,000 mg/L. A ten percent signal suppression results when sulfate is present at 200 mg/L.

In this study all atomic absorption measurements were made with a Perkin-Elmer Model 360 Atomic Absorption Spectrophotometer equipped with a Perkin-Elmer HGA 2100 Graphite Furnace. The spectrophotometer conditions were: Silt--Alt. 0.7 nm; Mode--TCl; Exp--15X. A Perkin-Elmer molybdenum hollow cathode lamp was operated at 30 ma. The monochromator was set to pass the 313.3 nm resonance line of molybdenum. The graphite furnace conditions were: Dry--50 sec at 120°C; Char--40 sec at 1,800°C; Atomize--8 sec at 2,800°C. The argon purge gas was set at continous flow (normal) at "20" units on the flow

meter. Aqueous standards containing 0, 10, 20, 30, and 50 µgMo/L were prepared from MoO_3. Aliquots of 25 µL were used for standards and samples. A 25 µL aliquot of 50 µgMo/L will yield an absorbance of about 0.12.

It is necessary to emphasize again the importance of testing for interferences when this method is used. Highly saline or industrially contaminated waters represent a significant potential for inaccurate analysis when this method is used without prior validation.